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PHYSIOLOGICAL REVIEWS

VOL. X APRIL, 1930 No. 2

THE PRESENT STATUS OF THE THEORIES OF NARCOSIS

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Fortunate indeed is a reviewer who finds as a guide to the literature of the subject and the development of the scientific ideas of which he has to treat, a book as complete and as thorough as Hans Winterstein's *Die Narcose* (189). Though few of his papers, perhaps, will be referred to in the course of this necessarily brief review, tribute should be paid not only to his book but also to his constant critical and constructive work in this field since 1900. Two other reviews have appeared, those of Kochmann (74) and of Hans Meyer (108), both of which are more biased in their use of the literature than is Winterstein.

The author has only considered it incumbent upon him to present in brief fashion such theories of narcosis as have been disproved and to show how they have influenced subsequent thought while dealing at greater length with those theories which are as yet the subject of dispute. These theories he has tried to present fairly without bias, leaving any personal opinions to a concluding paragraph. Nor has the author attempted to cite all the available literature he has read but only such papers as he considers of value in showing the development of the theories.

Claude Bernard (8) in the course of his studies of such drugs as caused anesthesia or depression of the central nervous system observed that in larger doses or acting for a longer period of time many of these drugs caused a similar, reversible depression of all living cells. Such drugs he termed narcotics and the process narcosis, from the Greek, ναρκαω (I am stupefied). Yet even from the first Bernard recognized that this group could not be made to include all drugs whose exhibition decreased sensation and that morphine and other alkaloidal depressants lay without the true narcotic group. Hence it is unfortunate that the word narcotic has in popular and legal language to-day gained a connotation drawn from the effects of opium, its alkaloids and cocaine.

The drugs to which we shall refer as narcotics are those that are

frequently classed as the general anesthetics and the hypnotics, though, of course, many of them are not commonly employed in medicine. They are substances which depress the central nervous system when present in a certain concentration and when their concentration falls beyond a certain point the cells are restored to their former activity. In the purest members of the group they leave little trace of their presence either in metabolic or histological changes in the cells after they have disappeared.

The search for the causative mechanism of narcosis and of its real raison d'être has led to the examination of the effect of narcotica on all types of living forms and on all types of tissues. The reason for this is manifest in the conception that narcosis is a general phenomenon manifested by living matter in response to the presence of an adequate amount of a narcotic within the cell.

Those living forms or tissues which when stimulated lead to a visible reflex response or one which can readily be made visible by our modern mechanical or electrical methods are much more readily affected by narcotics. The contractile movements of Gonionemus are depressed while its ciliary movements are not. It is not necessarily the case that the changes produced by an anesthetic in nerve cells are the same as those which are produced in other cells. Indeed Meyer (108) tacitly denies, as we shall see, that they are the same. Those cells or organs whose life can only be judged by the enzymatic processes of respiration or metabolism require higher concentrations of narcotics as a rule to lead to a measurable depression. None of us will probably deny that chemical, or at all events physico-chemical, processes are involved both in the responses of nerve cells and of other cells, yet they do not appear to be the same. A certain concentration of narcotic as we shall see decreases the contraction of muscle; a higher concentration depresses muscle oxidation. We are in this case using two different criteria for narcosis. When, further, the conception of narcosis is extended to such chemical or physico-chemical activity as the action of isolated ferments, or, further, to the effect of narcotics as shown by a depression of the ability of charcoal to lead to the oxidation of sugar or amino-acids adsorbed on it, it is obvious that the mechanism may or may not be the same as in the central nervous system. Here beyond doubt the explanation must lie in a very elemental physical process such as adsorption, but whether such a primary process is adequate to explain the facts obtained from the study of the simple anesthesia of men and animals is a question which it is part of the purpose of this study to disclose.

The following table makes it evident that higher concentrations of a narcotic are required to depress the simpler forms and expressions of life than the higher ones.

FORM OR TISSUE	AUTHOR	NARCOTIC	CONCENTRATION (G. PER 100)
Man	P. Bert (9)	Chloroform	0.008 in air
Dog	P. Bert (9)	Chloroform	0.009 in air
Polywog	Overton (130)	Chloroform	0.016 in water
Fish	Joachimogliu (65a)	Chloroform	0.030 in water
Fundulus embryo	Loeb and Wasteneys (94)	Chloroform	0.070 in water
Insects	Overton (130)	Chloroform	0.016 in water
Worms	Overton (130)	Chloroform	0.03–0.05 in water
Paramoecia	Fühner (44)	Chloroform	0.12
Frogs	Winterstein	Ethyl Urethane	0.5 perfusion of cord
	Krogh (76)	Ethyl Urethane	0.43 reflexes gone
Polywogs	Baum (7)	Ethyl Urethane	0.36 in water
	Overton (130)	Ethyl Urethane	0.3–0.4 in water
	Krogh (76)	Ethyl Urethane	0.48 in water
Fish Cyprimus	Krogh (76)	Ethyl Urethane	0.45 in water
Anthropoda Cyclops	Krogh (76)	Ethyl Urethane	0.6–0.7 in water
Molusces Mytilus	Krogh (76)	Ethyl Urethane	1.0 incomplete in water
Worm Nereis	Krogh (76)	Ethyl Urethane	1.0 incomplete in water
Medusa Gonionemus	Loeb and Wasteneys (94)	Ethyl Urethane	1.7 incomplete in water
Red Cells Goose	Warburg (168)	Ethyl Urethane	0.33 in water (50–70 per cent oxidative decrease)
Fundulus division	Lillie	Ethyl Urethane	1.5–1.75 in water
Yeast Cell	Dorner (30)	Ethyl Urethane	0.40 in water (fermentative decrease)
Acetone Yeast Extract	Warburg (175)	Ethyl Urethane	0.68 in water (almost complete fermentative decrease)
Liver Marcerate Suspension	Warburg (169)	Ethyl Urethane	0.36 in water (oxidative depression 50–70 per cent)
Watery Extract	Warburg (169)	Ethyl Urethane	0.74 in water (oxidative depression 50–70 per cent)
Algae	Warburg	Ethyl Urethane	1.95 in water (assimilation depression 50 per cent)
Algae	Warburg	Ethyl Urethane	5.85 in water (respiration depression 50 per cent)

In part adapted from Winterstein (189).

In the work of Rothert figures may be seen showing the effect on microorganisms.

Soon after the discovery of anesthesia several writers thought that this phenomenon was to be explained by mechanical changes in the blood supply to the brain but these ideas did not long survive adequate experimental examination and from the time of Carl Binz, 1877 (14) no such theory has been advanced.

I. The earliest biochemical theory of narcosis is that of Bibra and Harless (11), 1847, who made a series of experiments on frogs and mammals with ether, acetic ether, and ethyl chloride. They called attention to the fact that these and other anesthetic substances were not only good solvents for ordinary fat but also for the fatty substances in the brain and further suggested that the cause of narcosis was that the anesthetic dissolved out of the brain cells certain of its fatty constituents which were redeposited on the liver. Consequently they anesthetized two groups of animals of the same age and weight for hours with ether, and then determined the quantity of fat in brains and liver of the anesthetized and unanesthetized groups. They believed that their findings supported their theory but critical examination of their methods and results at once revealed that no proof had been presented.

In 1866 Hermann (58) came to a somewhat similar conclusion but in 1874 he expressed himself more cautiously, "Possibly it is these substances (lecethin, cholesterin and fat) for which these bodies (the anesthetic) are solvents, which are the site of action of the latter."

It may be noted that in 1908 Riecher (135) showed that during a chloroform anesthesia there occurred an increase of the fat in the blood stream and this has been noted by others. The theory of Bibra and Harless was important as precursor of the modern lipoid theory.

II. Precipitation theories. In 1867 and again in 1877 Ranke (133) called attention to the precipitation produced in clear filtered solutions of muscle and of nerve tissues by certain of the narcotica.

In 1875 Claude Bernard (8) wrote, "We, however, consider that a certain number of arguments taken from the exact analysis of the facts enable us to conceive in a sufficiently clear fashion the physico-chemical action which they (the narcotica) exert on the nervous elements. Our view is that this action consists in a semicoagulation of the substance of the nerve cell, a coagulation which will not be definite (final) that is to say, that the substance of the anatomical element will be able to return to its primitive state after the elimination of the toxic agent." This theory was suggested to his mind by a reversible rigidity which occurred in frog's muscles when placed in a chloroform solution and further owing to his observing that nerves placed in a chloroform solu-

tion became more opaque at the moment when their irritability was lost.

Carl Binz (14) in 1877 laid fresh sections of brain tissue in solutions of chloroform, chloral and morphine and reported that the cells became cloudy and the nucleus indistinct. The solutions employed were not isotonic with the tissues and the concentration of narcotic used was high. Ranke (133) 1877 could not, however, confirm this for morphine. He observed that the protoplasm of certain infusoria became darker and the protoplasmic movements slower in chloroform solutions but the infusoria might recover when placed in water. If, however, the protoplasm had become granular and the protoplasmic movements had completely ceased no recovery was possible.

The precipitating effect of chloroform on blood serum and protein solution was studied in some detail by Salkowski (142) and by Formanek (35). In 1904 Moore and Roaf (118) attacked with great thoroughness the problem of the precipitation of proteins by chloroform and other anesthetics. They showed that the addition of about 1 per cent of chloroform to blood serum caused an opalescence but no precipitate that could be seen under a microscope. This opalescence was reversible. With higher concentrations, 2 per cent and upwards, an irreversible precipitation was produced. Hemoglobin solutions made by laking blood corpuscles with water showed no stage of opalescence preceding the precipitation. Strangely enough they regarded their blood serum and hemoglobin solutions as being free from lipoids though in their second paper they admit the presence of 0.206 per cent lipoids in the serum. They found that ether gave a precipitate which was not as abundant as with chloroform: benzol and xylol also produced precipitates. Moore and Roaf considered that anesthesia must be produced by means of reactions between the narcotics and the proteins as these alone "are universally present constituents of animal and plant cells." The lipoids do not need consideration as they "do not occur in demonstrable quantities in by far the greater number of animal and vegetable cells." This statement seems counter to the belief of most modern students of the physico-chemical structure of living tissue. Save the opalescence the precipitation appears to be irreversible and according to Calugareanu (21) and others the opalescence is due rather to an adsorption of chloroform on the protein molecule than to an early stage of precipitation. Chloroform in the concentration in which it is found in the blood stream during narcosis does not cause any precipitation even after months (Salkowski, 140).

With the development of the study of enzymatic processes and of colloidal chemistry the question of precipitation produced by narcotics enters on a new phase in which the major stress is laid on the adsorption of the narcotic on cell constituents and precipitation is no longer regarded as the chief factor in the mechanism of narcosis. Consequently these facts will be considered in more detail later.

In 1912 Warburg and Wiesel (175) noted the parellelism between the depression of the oxidative activity of narcotics and the precipitation that was produced in yeast juice extract and that like their narcotic effects both the oxidative depression and the precipitation was more marked with the higher members of homologous series. The concentrations too were not higher in some cases than those required to depress the oxidation of the living yeast cells. The depressive concentration is, however, much higher than that required to produce anesthesia in polywogs: for ethyl urethane 5 per cent, for phenyl urethane 0.05 per cent, or 0.67 gram mol. per L. Battelli and Stern (6), 1913, again noted a parellalism between the precipitation of nucleoproteins and the depression of the oxidative enzymes, oxydones, that they obtained from several tissues but again the precipitations and depression of oxidation are not reversible and the concentrations far too high in comparison with those required for narcosis to lend any support to a theory which supposes an early stage of precipitation to be the cause of narcosis. Indeed some of the narcotics were not sufficiently soluble to produce precipitation at all; even with chloroform the solution had to be hypersaturated.

The similar experiments of Freundlich and Rona, 1917, Meyerhof, 1918, and others will be considered in connection with the adsorption theory.

In all the evidence produced in regard to the precipitation of proteins by narcotics, the concentrations required are much higher than those required to produce narcosis in animals or of the reflex reactions of tissues or cells. The precipitations are in most cases irreversible and consequently can not be the cause of the reversible phenomenon of narcosis, while the adsorption which probably precedes the precipitation may be the cause. It is, however, not impossible that the toxic effect in many tissues of narcotics in high concentrations may be accounted for in part by precipitation produced intracellularly or in the cell surface.

III. The dehydration theories. In 1882 Dubois (31) suggested that the cause of anesthesia was the loss of water from the anesthetized

cell produced by the action of the anesthetic upon it. This was a result of some experiments in which he placed parts of Echevaria or other plants in a closed vessel and exposed them to the vapours of chloroform. After an interval the plants transuded water and large droplets appeared on the epidermis. He noted that ether and alcohol have the same effect as does low temperature. Consequently he suggested that the loss of water was the cause of narcosis. He did not prove that the phenomena he observed were reversible. The anesthetic concentration must have been high and probably caused death of the cells.

Stephanowski (142) in 1902 observed that if Vorticella were exposed to the vapours from watery solutions of chloroform, or alcohol, at first a greater activity of the contractile vacuole was evident. Then the activity became less and finally it became inactive and other vacuoles appeared in the protoplasm. At this stage removal of the chloroform leads to a reversal of these changes. But if the chloroform be left the protoplasm becomes more transparent and later the Vorticella diminishes in volume. Hence he agrees with Dubois that a separation of water from the protoplasm represents the stage of narcosis.

Knaffl-Lenz (71) in 1918 performed two series of experiments. In the first he showed that placing erythrocytes in saline containing low concentrations of alcohol did not cause hemolysis but rather caused a shrinkage of the cells and protected them to a certain extent from hemolysis due to an hypotonic saline. In higher concentrations it, of course, caused swelling and hemolysis. The same results were obtained with urethane N/50 salicylamide and benzamide N/100 and diethylether N7./5. From these experiments he was led to the study of gelatine plates which when exposed to narcotics lost water but increased in weight. Petrolether, however, had no effect.

Kochmann (75) and his pupils in 1921–22 then carried out a series of researches in which they studied the swelling of fibrin particles prepared according to the method of M. H. Fisher. The swelling produced by distilled water or by 1/100 hydrochloric acid was decreased by narcotics. These, therefore, either produced a dehydration or prevented hydration. The effect was reversible.

It will be noted that in lower concentrations these narcotics aided swelling and that these lower concentrations approach more closely to anesthetic concentrations which for chloroform for example in the blood stream is approximately 40 mgm. per 100.

They further studied the effect of these narcotics on frog's gastroc-

nemii, ascertaining in each case the concentration which made the muscle non-irritable and also its change in weight.

NARCOTIC	CONCENTRATION JUST NARCOTIC FOR MUSCLE		LOSS OF WEIGHT TO NARCOSIS IN PER CENT
	In millimols	In per cent	
Chloroform	4.1	0.049	1.9
Chloral hydrate	6.1	0.1	10.7
Amyl alcohol	20.0	0.15	4.3
Butyl alcohol	66.6	0.5	5.4
Diethyl ether	190.0	1.4	2.6
Ethyl methane	250.0	2.0	3.0
Propyl alcohol	250.0	1.5	3.5
Ethyl alcohol	1000.0	4.6	5.2
Methyl alcohol	2000.0	6.4	4.4

The changes are reversible. Here too a dehydration occurred but though Kochmann might regard the losses in weight fairly equal for biological experimentation, an increase from 1.9 to 10.7 seems rather great.

Kochmann further considers that such a dehydration or a lessening of the dispersity of the colloid particles can easily be brought into harmony with the decrease in permeability which many authors suppose is produced by narcotics.

Consequently Kochmann considers that narcosis can be supposed to be due to certain preceding processes and certain necessary mechanisms as follows. Preceding processes: lipoid solubility, water solubility, decrease in the "haft-intensity" of their watery solutions, adsorption on the cell lipoids or by other ways, may make it possible for the narcotics to be carried to or penetrate into the cell. Here the narcotics produce a reversible dehydration of the cell colloids or hinder their swelling thus decreasing the permeability of the cell membranes and this in turn leads to a decrease or cessation of their metabolism and cessation of their function or narcosis. This is the necessary process but as Winterstein (189, p. 400) so clearly points out then any process of dehydration must lead to narcosis or cessation of function which is certainly not the case; indeed in frog's muscle the opposite may be clearly shown. The muscles of curarized frogs which have received subcutaneous injections of glycerine become highly irritable due to dehydration (Langendorf, 80).

IV. Water solubility. In 1893 Richet (136) called attention to

the fact that in many cases the lower the solubility of a narcotic in water, the greater its narcotic strength. This is well seen in homologous series. Fühner (44) again called attention to this phenomenon and both its truth and its fallacy may well be illustrated by the following table from Fühner to which I have added the columns headed "order."

	WATER SOLUBILITY		NARCOTIC CONCENTRATIONS	
	Mols. per L.	Order	Mols. per L.	Order
Chloral hydrate	4.040	1	0.030	5
Isopral	>0.104	3	0.009	11
Chloretone	0.028	12	0.005	15
Methylene chloride	0.209	2	0.016	7
Chloroform	0.063	9	0.007	13
Tetrachlormethane	0.040	11	0.003	16
Methylene chloride	0.209	2	0.016	7
Methylene bromide	0.065	8	0.014	8
Chloroform	0.063	9	0.007	13
Bromoform	0.005	18	0.001	18
Ethyl chloride	0.088	6	0.046	2
Ethylene chloride	0.075	7	0.031	4
Tetrachlorethane	0.011	16	0.002	17
Pentachlorethane	0.0006	19	—	—
Hexachlorethane	0.00007	20	—	—
Methyl iodide	0.100	4	0.064	1
Ethyl iodide	0.025	14	0.012	9
Propyl iodide	0.005	17	0.005	14
Propyl bromide	0.022	15	0.008	12
Isopropyl bromide	0.026	13	0.010	10
Ethyl chloride	0.088	6	0.046	2
Ethyl bromide	0.093	5	0.033	3
Ethyl iodide	0.025	14	0.012	9
Propyl chloride	0.051	10	0.017	6
Propyl bromide	0.022	15	0.008	12
Propyl iodide	0.005	17	0.005	14

If one looks at any homologous series such as the second three methylene chloride, chloroform and tetrachlormethane, there is a decrease in

solubility and an increase in narcotic strength. If, however, one takes the whole list and assigns to each member its order in water solubility or in narcotic concentration as I have done, no parellelism is evident. Chloral hydrate is most soluble but fifth in narcotic strength; isopral third in the one case and eleventh in the other. Obviously water solubility must be a factor, else how could the narcotic reach the cell? Water insoluble, indifferent substances, like paraffin, are not narcotics. Yet water solubility can not be the sole or even the predominant factor.

V. THE ASPHYXIAL THEORY. In 1900 Winterstein (179) working in Verworn's laboratory performed an experiment which formed the basis for the asphyxial theory of narcosis. This original experiment was performed with carbon dioxide. In 1902 (180) he repeated the experiment with chloroform as follows: A frog was given strychnine and stimulated till exhausted. It was then perfused with saline free from oxygen and repeatedly stimulated, so that any supposed oxygen stores were exhausted, and the waste products formed were washed away. Then it was perfused with oxygenated blood-saline containing chloroform, thus giving the frog (or its cord) a chance to take up and bind oxygen if it could do so in the presence of the narcotic. Then oxygen-free saline was perfused to wash away the chloroform. No sign of recovery was shown till it was perfused with blood saline when tetany soon occurred. It was thus proved that a cell under a narcotic could not take up and store oxygen. Similar experiments were performed by Frölich (40) on nerves and Nagai (121) on ciliated epithelium. These experiments fitted in with Verworn's biogen hypothesis and led to his proposing a theory of narcosis in 1903. The biogen theory of Verworn presupposed that oxygen was built into the protoplasm molecule and thus retained until needed in metabolism. The asphyxial theory is, best perhaps, stated by Verworn (165) in a brief paper in 1909. In this paper he does not refer to it as a theory but rather as a statement allowing the process of anesthesia to be approached more closely and enabling certain suggestions to be made.

He considers that the above experiments and others showed that during anesthesia the oxygen absorbing activities of the cells were lost. He thought that the further experiments of Bondy (15) and Heaton (56) showed that oxygen disappeared from the cells during narcosis at the same rate as before narcosis and hence that an asphyxia must occur as a result of prolonged narcosis and considered that a suggestion of the effect of this asphyxial condition was to be seen in the appearance of acetone and lactic acid, both products of incomplete combustion, after prolonged anesthesia in man.

The experiment of Heaton (56) in which he placed a pair of frog's sciatic nerves in a narcotic solution and stimulated one of them above the narcotized area with the result that it failed to respond before the other, Verworn considers as showing that catabolism not only proceeds during narcosis but can be increased by doing work. Further he considers that other experimentation has served to confirm his suggestion that catabolic loss of oxygen proceeds normally during narcosis but not the taking up of oxygen and that hence the longer the narcosis in any case the greater the asphyxiation produced. This secondary change alone may produce death in prolonged anesthesia. He suggests then that the atomic groups in the cell which transfer the oxygen to the cell may enter into a loose chemical union with the anesthetic solute and the mechanical presence of the narcotic molecules may prevent any rapid catabolic process which would be evident as a visible response.

Though in the light of our present knowledge of cellular oxidations the experiments are capable of quite different explanations than those advanced by the experimenters and Verworn the theory stimulated much further research. Much of the detail it is not necessary to follow, nor have we adduced all the evidence available at the time of Verworn's paper quoted above. Certain ideas, however, we find reappearing in varying form almost to the present and consequently we must follow certain of the trails followed by anesthetic research.

Mansfeld (102) in 1909 adduced evidence that polywogs were narcotized by lower concentration of paraldehyde when in an atmosphere free from oxygen and proceeded from this to the theory as result of the work of one of his pupils, Hamburger (50), that narcotics prevented the cell lipoids from absorbing oxygen. The original experiment could not be confirmed by Kisch (70) but he thought that many narcotics decrease the oxygen absorption by living cells.

The physical proof that narcotics decreased the solubility of oxygen in lipoids, was correctly and roughly demolished by Winterstein (183). Yet the observation of Wieland 1922 that nitrous oxide was a better anesthetic when oxygen want is present has been amply confirmed by the work of Green and Currey (48) and of Brown, Lucas and Henderson (18). But here as in many of the other experimental observations adduced in support of the asphyxial theory we are merely dealing with two chemical factors, lack of oxygen and presence of narcotic which augment each other.

Wieland's (178) attempt to separate the anesthetic gases, nitrous oxide, ethylene and acetylene from the other anesthetics has been com-

pletely disproved by Graham (47) who has shown that even such an anaerobic process as the contraction and conduction in frog's muscle could be depressed by sufficiently high pressures of these gases. Contraction, however, could not be completely abolished by these gases without producing irreversible changes in the muscle.

Bürker (19) suggested that the oxidation of a narcotic or a part thereof by the cell used up the available oxygen. This suggestion is not of course tenable as an explanation of narcosis as some narcotics (at all events N_2O) are certainly not oxidized and others only in minute quantities. Yet this small oxidation may account for some of their toxic properties (Lucas, 97).

The main theses of Verworn were destroyed by the following types of evidence.

a. Overton (130) anesthetized polywogs for days, Krogh (77), 1910, did the same with frogs, using ethyl urethane. Ellis (33), 1923, added evidence of even greater moment when he showed that pigeons could withstand anesthetization with barbital for days. Certainly in the rapid metabolism of birds Verworn's postulated asphyxia should be evident. Winterstein (184) also narcotized frogs for days with urethane and removed the narcotic by perfusing with oxygen-free saline. The frogs became active in one or two minutes. Here it is evident that an asphyxial process could not have been produced as the oxygen free saline would only tend to increase the asphyxia.

b. Warburg (167) showed that the fission of sea-urchins' eggs was readily inhibited by narcotics while oxidation was little or not affected. This was confirmed by Loeb and Wasteneys, 1913, who also showed that the movements of Fundulus embryos are decreased before their oxidations, while with Gonionemus there was more decrease in oxidation. Lillie also showed that narcotics decreased the cleavage process of Arbacia eggs which cyanides, in concentrations which decrease the oxidation, do not do. Issekutz (62), 1918, showed that the oxidations of polywogs were not affected by narcotics but greatly by cyanide which did not cause paralysis. Weizsäcker, 1917, showed that 10 to 12 times as much cyanide was necessary to abolish the contraction of muscle as to inhibit its oxidations while only $\frac{1}{2}$ to $\frac{1}{3}$ as much urethane was required to make them non-irritable as was required to abolish the oxidation. Usui (161), 1912, thought he proved the same for the central nervous system. Winterstein (184) showed that the amount of alcohol which depressed the central nervous system led to increased oxidation.

These facts distinctly show that oxidative processes and narcosis are separate phenomena.

c. Warburg and Wiesel (175), 1912, showed that the reproduction and fermentation of yeast were hindered by narcotics yet oxygen is not needed by yeast for these processes. Winterstein (183) showed that Ascaris which does not require free oxygen is paralyzed by chloroform and ether. Veszi (166), 1918, showed that obligate anaerobic bacteria could be anesthetized. Here we see processes that are non-oxidative depressed by narcotics, consequently narcosis cannot be due to any form of asphyxia in these cases and hence can not form the basis of a general theory of narcosis.

Yet the appearance of such waste products as acetone and lactic acid after narcosis and such observations as the following have served in a sense to keep alive the idea that narcosis in mammals is necessarily accompanied by defective oxidations.

Thomas (144), 1898, was one of the first to study the changes in acid base equilibrium in the body during narcosis and found a decrease in blood alkalinity with chloroform and alcohol but not with ether. Fatty changes in many organs after chloroform were noted by many observers, Rubow (138), for example. Bang (4) noted a disappearance of liver glycogen during narcosis. Lesser (84) showed that lack of oxygen could produce similar changes.

Loewi (96), 1907, summing up the metabolic disturbances after chloroform suggests that they may all be considered as fully analogous to the effects of cyanide, phosphorus, etc., which decrease the oxidative ability of the cells. Oppermann (128) made a careful study of the production of hyperglycemia by narcotics and attributed it to disturbed oxidation. Pawel (131) again as the result of his experiments agreed that the fall in blood alkali, increased protein catabolism, decreased temperature and presence of acetone bodies in the urine could all be ascribed to the decreased use of oxygen produced by the presence of an anesthetic. Joannovics and Pick (66) also called attention to an inhibition of oxidation in the liver produced by narcotics.

In more recent times the changes in acid-base balance in the blood stream have been followed by the greatly improved modern methods of analysis. We may quote Cullen, Austin, Kornblum and Robinson (26) who showed that ether anesthesia caused a marked fall in both pH and alkali reserve leading to an uncompensated alkali deficit. Morris (120) showed the same occurred with chloroform. Leake (82) showed that light anesthesia with ethylene produced little such change, though if the oxygen supply were necessarily decreased to produce deep anesthesia, the same type of change occurred as with nitrous oxide. Brown

and Henderson (17) then showed that during surgical anesthesia with propylene in dogs, there was no such shift in pH and alkali reserve; the only change being a slight increase in both and no change in blood sugar. The same observations have been made using cyclopropane (Lucas and Henderson, 98). Consequently we may infer that the change in acid-base equilibrium is not a necessary accompaniment of narcosis but is due to the peculiar properties of certain of the anesthetics and possibly is due to the intracellular oxidation of small amounts of the absorbed anesthetic or in the case of nitrous oxide and ethylene to the lack of adequate oxygen to maintain normal cell metabolism.

We have thus far been following in a sense an historical order in considering the theories of narcosis but from this we shall depart to refer to the theory proposed by Traube.

VI. TRAUBE'S THEORY. This theory developed a hydra-headed character and though its author finally admits that one head has been deprived of its support he nevertheless claims that the theory retains some truth in view of the existence of the other head. Yet we will see that it too is no longer viable. Nevertheless the theory has been of importance in the development of the theories of narcosis.

In 1904 Traube (147), noticed in his experiments on osmosis that those substances which lowered the surface tension of water passed most rapidly into the cell. This was certainly true of the substances which he employed. Surface tension then became for him an important measure of the ability of a substance to pass a cell membrane. Further his studies had led him to notice that both the ability to lower surface tension at a water-air interface and ability to penetrate increased in homologous series in a relation that could be expressed by 1:3:9, etc. Now it had long been known that the narcotic effect of homologous series of narcotics increase and often in a similar relationship hence a relationship between the ability to lower surface tension or the capillary activity of a substance and its narcotic capacity was suggested (148). Independently of Traube, Billard and Dieulafé (13) suggested a relationship between capillary activity and narcotic strength.

As a result of his experiments he formulated in 1910 (149–150) a theory expressing this relationship.

According to the Gibbs-Thompson theorem, the more a substance lowers the surface tension of its solution, the more abundantly it must collect on the surface. Traube regarded this fact from a somewhat different angle, when he suggests that the more a substance lowered the surface tension the less it clung (haftet) to the main mass of the

fluid or was retained by it, consequently the more it lowered the surface tension the less the force (Haft druck or Haft intensität) "clinging intensity" and this could be measured by its capillary activity at an air-water interface. In 1913 Traube (151, 152) presented tables in which he gives the capillary activity of a series of alcohols at 15°C. measured with a stalagmometer and compares the molecular concentration of their isocapillary solutions and with their narcotic effect as ascertained by Overton on polywogs, and Overton's distribution coefficients.

	PARTITION COEFFICIENT OIL : WATER OVERTON	NARCOTIC CONCENTRATION MOL. PER LITER	CAPILLARY ACTIVITY WATER = 91.5 MM.	MOLAR CONCENTRATION ISO-CAPILLARY SOLUTIONS	PARTITION COEFFICIENT WROTH AND REID
Methyl alcohol	50: ∞	0.57	88.6	14.0	0.0096
Ethyl alcohol	1:30	0.29	84.0	5.0	0.035
N-Propyl alcohol	1:8	0.11	74.0	1.6	0.156
I-Butyl alcohol	6:1	0.045	56.5	0.46	—
N-Butyl alcohol	∞:12	0.038	—	0.45	0.588
I-Amyl alcohol	∞:2	0.023	37.4	0.14	—

I have added the partition coefficients according to Wroth and Reid (190).

It is natural that Traube regards capillary activity a better measure of narcotic efficiency than the partition figures of Overton which we will subsequently see are not of great accuracy. Of course considerable lack of agreement between capillary activity and narcotic concentration is evident when the figures are regarded more closely. But in a rejoinder to a criticism by Höber he points out that "Haftdruck" to water and to lipoid are not of the same value (157). And in another article he states that the lipoid content of brain cells must have an influence on the absorption of narcotics. In other words, that an air-water interface may not present the same features as a water lipoid one.

He received, however, a certain amount of support from von Issekutz (62), 1918, who considered that his experiments showed that capillary activity varied in the same sense with temperature as their narcotic effectiveness. His figures have not, however, been generally accepted. Kisch (69) showed that yeast cells were permanently damaged when a narcotic was added to their watery solution in such concentration as to lower its surface tension to less than half of that of water. Czapek (27), 1918, also showed that the cells of Echevaria showed exosmosis of tannin when the capillary activity of the surrounding water was lowered by narcotics to 68 or 69 per cent of its original value. This work has been attacked by Vernon (163).

Traube's studies then led him to consider the effect of changes of surface tension on colloids and gel formation (153–155) and led him in 1919 to suggest that the increased dispersion produced in colloid solutions by small amounts of narcotics corresponded with the excitement phase, while the reverse would form the cause of narcosis (154).

In 1921 Joachimoglu (65) proved that a long series of halogen substituted hydrocarbons, including such well-known narcotics as chloroform and ethyl chloride showed no capillary activity as measured by Traube's method and in the same year Fühner (42) showed that the same was true of the saturated hydrocarbons, pentane, hexane, etc.

Traube's answer consisted in the assertion that chloroform does not exist in water in true solution but in a colloid form basing his statement on the experiments of a pupil who thought that he had observed the Tyndall phenomenon in chloroform solutions (157).

In 1923 Bose (16) proved that this suggestion was false and that chloroform existed in true solution.

Traube in conjunction with a pupil, Tomita (146), in 1924, had transferred his studies to the absorption of solutions containing narcotics by gelatine gels, first pure and then gels containing cholesterin and lecithin as well. These experiments forced him to the conclusion that the presence of lipoids is of great importance for the penetration of narcotics (156). Finally he is forced to admit that while strongly water soluble narcotics depend for their penetration on capillary activity, the true narcotics are more dependent on the presence of lipoids. "Haftdruck" "clinging-intensity" to lipoids and to water are usually the reverse of one another. The "clinging-intensity" is then of importance merely for the capillary active highly water-soluble narcotica (156–158).

Certainly if the whole narcotic series be regarded from the standpoint of water solubility a border line between his two groups is hard to find and water solubility as we have seen does not afford a criterion of anesthetic potency. Nor in view of the fact that chloroform and many other narcotics are not capillary active does capillary activity form a criterion. Further, there are many highly capillary active substances such as peptones and soaps which do not act as narcotics. However it must not be forgotten that the criterion of the effect of a narcotic at an air-water interface does not necessarily give us any indication of the effect that it will produce at a water-cell interface. While Traube's theory, as he in essence admits, falls to the ground, it is of importance in connection with the theory of adsorption and also because of the admission to which he was forced by experiment of the importance of lipoids in connection with the penetration of anesthetics.

MEYER OVERTON. THE LIPOID THEORY. As we have learnt above Bibra and Harless and later Hermann suggested a relationship between the fatty constituents of the tissues and the action of anesthetics. Overton suggested the word "lipoid" as a facile and all-embracing term for not only the true fats but also and especially those more complex fatty-like bodies, lecithin, cholesterin, protagon, etc. The term will be employed with this meaning.

Pohl, (132) in 1891, published experiments which showed that the red blood cells took up more chloroform than the plasma and attributed this to their containing the lipoid bodies, lecithin and cholesterin. He also found more in brain than other tissues.

These findings of Pohl (132) and the analyses made by Nicloux (122) both of which will be discussed later are referred to by Meyer in his original papers (105) and in his chapters on the theory of narcosis in the Gottlieb-Meyer text book (107).

In Meyer's laboratory since 1895 experiments with various narcotics had been in progress, while in Overton's biological laboratory experiments were being carried out on the permeability of living cells for a great variety of substances including the narcotics. Both observers had by 1899 formulated a theory of narcosis, which a mutual friend found identical. Meyer (105) then in 1899 published a general statement of the theory supported by a paper by his pupil Baum (7). Overton's publication (130) was deferred till 1901. In 1901 Meyer (106) published a second paper which explained some of the apparent discrepancies in Baum's work. In 1921 and in 1923 papers appeared by Kurt H. Meyer (109) and two different collaborators. Meyer has set forth the theory in successive editions of his text book and in a review in Bethe's Handbuch (108). In all cases these five papers by Meyer, Baum and K. H. Meyer form the main body of proof, though he acknowledges the important support received from Overton's work and refers to the Pohl Nicloux and other papers dealing with distribution of anesthetics in the body.

We will set forth the evidence and the theory as briefly as possible following closely Meyer's presentation but before doing so it is important to point out that Meyer claims validity for his theory only as applying to what he terms "reflex life of all living forms" (108). He therefore either tacitly denies that the word narcosis should be applied to a reversible depression of metabolism or that such narcosis may or does depend on a different process than that which inhibits the expression of movement and excitation.

Meyer sets down his fundamental propositions as follows:

1. All chemically indifferent bodies, which are soluble in fats or fat-like substances (lipoids) must act as narcotics to living protoplasm in so far as they can become distributed within it.

2. Their action must appear first and most markedly in those cells in whose chemical structure these fat-like substances predominate and form peculiarly essential participants in the cell function.

3. The relative degrees of activity of such narcotics must depend upon their mechanical affinity to the fat-like substances on the one hand and to the other constituents of the body, i.e., water on the other. Consequently on the partition coefficient which determines their distribution in the mixture of water and fat-like substances.

These statements met with the full approval of Overton.

METHODS. Both Meyer (105), Baum (7) and Overton (130) determined the anesthetic potency of narcotics by placing groups of polywogs in dishes containing varying strengths of the narcotic under examination and determining the concentration necessary to cause the loss of certain simple reflexes. In the table below these are expressed in mols. per litre as this furnished a common basis for comparison which percentage strengths would not do.

Meyer determined the partition coefficient by dissolving a certain quantity of the narcotic in water (or in oil) and then adding an equal quantity of pure olive oil (or of water) and agitating thoroughly in a closed glass cylinder. After shaking and standing till the temporary emulsion broke and a clear line of separation appeared, the watery part was analysed to find its content in narcotic. The difference between the original value and the new gave the amount which had passed into oil. Olive oil was used as it was impossible to employ brain tissue or even the extracted and denaturized brain lipoids. These procedures naturally limited the choice of anesthetics but Meyer used typical members of nearly all the narcotic groups of chemicals save the gases. Overton experimented with a larger range of substances, employing frequently all the available members of an homologous series. Unfortunately he was unable to ascertain the true distribution coefficients in many cases but inferred this ratio from the solubility in oil and in water separately. Wroth and Reid (190) have shown the fallacy of such a procedure and that determinations can only be made by a method such as that employed by Meyer. The results obtained by Meyer are seen in the following table which differs from that of Baum as corrections have been made due to Meyer's later work.

	$\frac{\text{C-LIP.}}{\text{C-WATER}}$	NARCOTIC CONCENTRATION IN MOLS. PER L.
Trional	4.4	0.0013
Tetronal	4.0	0.0018
Butylchloral	1.6	0.002
Sulphonal	1.1	0.006
Bromalhydrate	0.7	0.002
Benzamide	0.6	0.002
Triacetin	0.3	0.01
Diacetin	0.23	0.015
Chloralhydrate	0.22	0.025
Ethylurethane	0.14	0.025
Monoacetin	0.06	0.02
Methylurethane	0.04	0.4
Alcohol	0.03	0.5

Certain discrepancies which appeared in Baum's work called attention to the fact that the partition coefficients and the narcotic potency had not been ascertained at the same temperature. This led to Meyer's second paper (106) which showed that with certain narcotics the oil-water coefficient and the potency increased at higher temperatures and in other cases both decreased. As we shall see, this, if true, furnishes the strongest support to this theory or rather acts as the most difficult obstacle to the acceptance of other theories.

In these experiments Meyer changed the temperature of the frogs very slowly taking up to two days to raise the temperature from 3° to 30°. This precaution has been neglected by all who have attempted to repeat his results and some of the paradoxical results seen by other observers are doubtless due to the change of temperature being too rapid, in many cases taking little more than one hour.

	NARCOTIC CONCENTRATION AS DILUTION OF NORMAL SOLUTION		PARTITION COEFFICIENT OIL/WATER	
	3°C.	30°C.	3°C.	30°C.
Salicylamide	1:1300	1:600	2.23	1.40
Benzamide	1:500	1:200	0.67	0.43
Monacetin	1:90	1:70	0.093	0.066
Ethyl alcohol	1:3	1:7	0.024	0.046
Chloralhydrate	1:50	1:250	0.053	0.236
Acetone	1:3	1:7	0.140	0.195

To a large extent the difficulties in determining the partition coefficient between oil and water can be eliminated in the cases of the volatile anesthetics as their tension in the blood depends directly on their partial pressure in the inhaled air (Bert, 9). Consequently Kurt H. Meyer (109) with Gottlieb-Billroth and later with H. Hopff determined the narcotic concentration in vapour form of a long series of narcotic substances.

ANIMAL	SUBSTANCE	L = SOLUBILITY COEFFICIENT	C = NARCOTIC CONCENTRATION IN VOLUME, PER CENT	C_{lip} = CONCENTRATION OF THE NARCOTIC IN MOLS. PER LITER LIPOID
Frog	Nitrogen	0.05	9000	0.18
	Methane	0.54	760	0.17
Mouse	Methane	0.54	370	0.08
	Ethylene	1.3	80	0.04
	Nitrous oxide	1.4	100	0.06
	Acetylene	1.8	65	0.05
	Dimethylether	11.6	12	0.06
	Methylchloride	14.0	6.5	0.07
	Ethylene oxide	31	5.8	0.07
	Ethylchloride	40.5	5.0	0.08
	Diethylether	50	3.4	0.07
	Amylene	65	4.0	0.10
	Methylal	75	2.8	0.08
	Ethylbromide	95	1.9	0.07
	Dimethylacetal	100	1.9	0.06
	Diethylformal	120	1.0	0.05
	Dichlorethylene	130	0.95	0.05
	Carbon disulphide	160	1.1	0.07
	Chloroform	265	0.5	0.05
Experiments on mice (Führer)	Pentane	37	13.2	0.19
	Benzol	240	1.2	0.12
	Chloroform	265	0.8	0.09

The narcotic concentrations are expressed in volumes per cent so that 370 means 3.7 atmospheres of partial pressure for methane; 80 means in an air-oxygen mixture containing 80 per cent ethylene. The solubility in oil was obtained by the observers and means cubic centimeters gas per 100 cc. fluid. This is expressed in the tables as L. If now the solubility coefficient L and the narcotic concentration C of the vapour or gas in volume per cent in an air mixture at 760 mm. is known,

the content of the narcotic in mols. in the lipoid of the narcotized cell may be calculated to give the last column as follows:

$$\text{C. Lip.} = \frac{1}{R} \cdot \frac{C.L.}{100}$$

where $R = 24$, the number of litres of a gas at 760 and 20°C. It will be noted that the range of variation for C. Lip. in mice is from 0.04–0.10 in the experiments of the authors, to 0.19 if Fühner's results are included. This is not a wide range in view of the possible errors and in view of the wide difference of 10,000 times in anesthetic potency.

Meyer and Gottlieb-Billroth call attention to their calculated figure for the molecular concentration of chloroform in the brain lipoid 0.05 ±0.01 and compare it with the analysis made on grey and white matter by Frisson and Nicloux (39) in four experiments on dogs which when calculated gives 0.04. A further table based on the experiments with polywogs according to Meyer and Baum, and Overton (though the approximate partition coefficients were only roughly made in some cases by the latter and have no claim to great exactitude) was made by K. H. Meyer and Gottlieb-Billroth and the narcotic concentration in mols. per litre lipoid calculated. Here the variations are from 0.001 to 0.27 though but four exceed the figure of 0.09 in the list of 39 and many of them are extreme members of the class of "indifferent" narcotica. This variation is not considered by Meyer (108) great in view of the part that their anesthetic concentrations vary from 0.3 to 0.000004 mol. per litre. To this may be added the following considerations.

1. It will be seen from the observations made on narcotics as a class in the introduction that they differ greatly in their chemical "indifference" if the word may be used. Only some 2 per cent of alcohol will be excreted unchanged. Some 1 to 2 per cent of chloroform or bromoform may be broken up in the body (Lucas, 97) and of such narcotics as ethylene and even more so cyclopropane and nitrous oxide little or perhaps none is attacked. It is well known that most of the hypnotics undergo change in the body. This will introduce a certain small change in the estimation of narcotic strength which will vary from anesthetic to anesthetic. Further, the criterion of anesthetic strength in polywogs or mice is at the best a rough one. The error on this score alone is some 10 per cent, perhaps even more. There is further the error arising in the estimation of water and oil solubility.

Perhaps even more important is the fact that olive oil is not a true representative lipoid and that further the lipoids or the mixture of lipoids is not necessarily a constant quantity in similar cells in different

animal forms, nor necessarily constant in any cell. Certainly if we judge from the effects of anesthetics the lipoid mixture must vary in cells let us say in the sensory area of the cerebral cortex from that contained in the respiratory centre and will naturally differ from that of liver cells amoeba, bacteria or plant cells.

2. Some critics like Moore and Roaf (118) have frankly denied the importance of the cell lipoids in cell function but we may take it that today there is very general agreement that lipoids exist in all cells in greater or less quantity and play an important if not perhaps the preeminent importance in cell function.

The following papers may also be quoted as evidence bearing on Meyer's postulates.

a. Mansfeld (101 and 103) showed that chloral and paraldehyde were more effective as narcotics in starved fat-poor animals than in fat ones. Cloetta (23) attacked his conclusions on the basis of experiments on normal rabbits and ones with a large fat content owing to arsenic poisoning but an examination of his protocols shows that the less fat animals were first and longer affected than the poisoned ones though the differences were slight.

Harass (53) studied a series of amides most of which, however, were not in their pharmacological effects typical narcotica, as the narcotic stage was preceded by convulsions in some cases in rabbits. The partition coefficients appear reliable. The threshold valves were obtained by placing frogs in solutions of varying strength.

	COEFFICIENT OIL/WATER	THRESHOLD VALUE
Valerdiethylamide	5.7965	1/171 = 0.00584
Valerdimethyl amide	0.4163	1/51 = 0.0196
Valeramide	0.313	1/9 = 0.111
Valerethyl amide	0.2536	1/50 = 0.02
Lactdiethyl amide	0.154	1/7 = 0.142
Sodium salicylate	0.018	1/4 = 0.25

Certainly the parallelism is not good as the valerian-ethylamide is too great an exception.

Frey (38) studied the parallelism between narcotic potency and oil-water partition coefficient by injection into rabbits.

	CHLORAL	CHLOROFORM	CHLORAN	DORMIOL	ISOPRAL	BUTYL-CHLORAL
Coefficient	0.22 (6)	14.78 (1)	1.97 (4)	0.515 (5)	9.59 (2)	2.25 (3)
Concentration	0.033 (6)	0.0009 (1)	0.00125 (2)	0.023 (5)	0.0014 (3)	0.002 (4)

The figures in brackets show the order of the coefficients and of the concentrations. No indication is given as to how the coefficients were ascertained. The parallelism is not exact but as a whole may be taken as supporting the theory.

Von Eeckhout (32) also obtained on small fish the narcotic concentration of a series of substituted ureas and their oil-water coefficients.

COEFFICIENT			THRESHOLD VALUE PER L.
Order	Value		
1	1.90	Methyl ethyl bromacetyl urea	0.00033
2	1.33	α Brom isovalerianyl urea	0.0005
3	1.05	α Iodisovalerianyl urea	0.00092
5	0.78	α Chlorisovalerianyl urea	0.001
8	0.6454	α Bromvalerianyl urea	0.001
10	0.487	Valerianyl urea	0.0011
4	0.9263	α Bromisobutyryl urea	0.0025
11	0.3718	α Brombutyryl urea	0.0028
9	0.63	α Bromisovalerianamide	0.005
7	0.689	Isovalerianyl urea	0.007
6	0.71	α Bromisobutyramide	0.025
12	0.24	α Brombutyramide	0.03

Here little parallelism is evident. Only 2 or 3 of these substances are narcotic for mammals and four are toxic and the others inactive or very weakly so. Evidence is also presented that several of these bodies are readily metabolized.

	LIGHT NARCOSIS	DEEP NARCOSIS	OIL/WATER COEFFICIENT	SOURCE OF SAME
	Mill. M. per L.			
Adalin	0.47	1.79	3.3	Redonnet
Bromnal	0.55	2.2	1.4	Cloetta
Neuronal	1.0	2.5	2.6	Redonnet
Chloroform	1.6	4.2	?	
Trional	2.0	5.1	4.46	Baum
Sulphonal	4.3	6.0	1.11	Baum
Dial	5.7	—	0.76	Tiffenau
Chloral hydrate	7.5	30	0.22	Baum
Paraldehyde	11.2	28	0.28	Cloetta
Ether	28.2	58	1.4	Cloetta
Amylene hydrate	33.0	50	?	
Urethane	33.0	56	0.14	Baum
Veronal	27.0	—	0.006	Tiffenau

Früh (41) experimented with a long series of narcotics on frogs immersed in their solutions. The above table shows no semblance of regularity but the method employed is not a favourable one.

In none of the above attempts to extend the Meyer-Overton data is there adequate evidence to either confirm or deny the theory. The original experiments should be repeated.

Aiello (1) calls in question the justice of the partition coefficient as determined for oil and water urging that oil and serum give very different values. The two narcotics he employed were trional and sulphonal.

	OIL/SERUM	OIL/WATER	
		Baum	Overton
Trional	0.27	4.45	16.00
Sulphonal	0.21	1.11	4.50

Quite apart from the serious discrepancy between the figures of Baum and Overton there is a great difference between his figures and theirs. But the determination of partition coefficients between oil and serum with the Meyer method would give rise to serious errors owing to the coating of oil globules during agitation with protein and the possible lipoid content of serum.

Criticism of the value of the partition coefficient as a measure of anesthetic efficiency has often been expressed. For example, Winterstein (189, p. 307) while admitting the general parallelism in Baum's figures calls attention to the fact that there is little difference in the narcotic concentration of trional 0.0018, butyl-chloral 0.0020 and bromal 0.0020 while the partition coefficients are 4.46, 1.59, and 0.66, i.e., 1: nearly $\frac{1}{3}$ and $\frac{1}{7}$; and in the cases of monacetin, potency 0.050 and coefficient 0.06, and methyl urethane, 0.40 and 0.04, again no proportionality is present. Warburg and Wiesel (175), considering figures given by Overton, note that isobutyl alcohol should be 180 times stronger than ethyl alcohol as judged by its partition coefficient yet it is only 3 to 4 times. According to Wroth and Reid's (190) more accurate figure of the partition coefficients it should be only 60 times stronger. Yet even here the difference is great. These criticisms Meyer has either anticipated by pointing out the sources of errors and the lack of identity of oil with brain lipoid as mentioned above and by rejoining that there is a parallelism even when one considers such extremely different narcotics as those in the table and by emphasizing the closeness of the data

furnished by K. H. Meyer for the calculated content in mols. per litre of brain lipoid.

b. Partition coefficient and narcosis with temperature changes. This important point has frequently been considered by investigations. Unfortunately no one has precisely repeated the Meyer experiments.

Moral (119) working with skeletal nerves thought that the effect of alcohol, chloral, monacetin and salicylamide changed with temperature with Meyer's change in partition coefficient.

Unger (160) confirmed Meyer for chloral and salicylamide on polywogs, but not on the fish Lenciscus.

Hartmann (54) working with Cladocera could also not confirm Meyer. His experiments, however, are not of great value.

Bierich (12) failed to confirm Meyer's results on polywogs with benzamide and salicylamide. The change of temperature was rapid. He did not try the others but remarkably enough confirmed the change of partition coefficient of these two substances with temperature. He used cod liver oil in place of olive oil.

Höber (60) tried the effect of narcotics on the irritability of muscle and conductivity of nerves at varying temperatures. His findings also are opposed to those of Meyer.

Redonnet (134) could not observe a corresponding change with temperature in the narcotic potency and partition coefficients of neuronal and adalin.

Dennecke (28) found both chloral and salicylamide more narcotic at 30° than at lower temperatures.

Knaffl-Lenz (72, 73) confirms the parallelism of the change of partition coefficient and narcotic potency with temperature for salicylamide and benzamide. He did not try the other narcotics in Meyer's series but used chloretone which like Meyer's acetone showed the reverse behavior in both cases. He also showed with these and other substances the capillary activity decreased in all cases with rise of temperature.

Von Issekutz (63) injected frogs with salicylamide, half the group were cooled in snow, the other half put in a thermostat and the groups were exchanged at the end of an hour. The depth of narcosis diminished on raising the temperature but with chloral and urethane they remained the same. But there was always less salicylamide in the brains of the cooled frogs though they appeared more narcotized. These experiments are of no value owing to the rapid changes of temperature.

Collet (25) found that if narcosis was judged by the swimming movements of Loligo and Gonionemus, the heart beat of Perophora, the Meyer rule that benzamide and salicylamide decrease in efficiency as

temperature rises hold, while chloral showed increased efficiency throughout the range of concentrations tested. With toad and frog tadpoles, gastrulae of Bdeloura and Arbacia this was true for chloral at all concentrations, but for benzaminde and salicylamide only at low ones. With cilia, tentacles, and chromatophores (including those of Loligo) the rule holds for chloral, but in the case of the other two the effect decreases as temperature falls. Here it is evident that reflex movements are differently affected from the movements of other simpler mechanisms. In its main essentials, we have agreement with Meyer.

The results of Winterstein (181) too who showed that the depth of alcohol narcosis of medusae, crustaceae and frogs increased on raising the temperature, may be quoted in support of the Meyer theory.

The evidence is here conflicting. Much of the work is of little value. An exact repetition of Meyer's work is urgently needed. On the whole the balance of evidence may be considered as in the favor of Meyer's theory.

c. As Meyer quotes in his writings some of the figures of Pohl (132) and of Nicloux (122) on the distribution of narcotics in the body, it seems pertinent to turn aside to the consideration of the results of such analyses. Surely from such experiments we should gain some information as to whether lipoid solubility influences the distribution of narcotics at all or not.

A great many workers (132, 29, 100, 2, 22, 126, 3, 57, 85, 117) have studied the distribution of various narcotics between the plasma or serum and corpuscles or between saline solutions and corpuscles, but unfortunately practically all observers have failed to make even lipoid analyses of the corpuscles and plasma used. Some indeed claim like Burkhardt (20) that the serum has a higher lipoid content than the corpuscles and as an ether extract of blood will contain any cholesterin present a further complication is added to the evaluation of the results as cholesterin is by no means a typical lipoid, yet in the blood it is present in relatively high amounts. Many of the figures might suggest that the lipoid content of the corpuscles was important, for instance, Apitz and Kochmann (2) who found 8.6 of chloroform in erythrocytes to 1 in the saline in which they were placed. But they also found 1.7 of alcohol to 1 under the same conditions. Now the partition coefficient oil/water for alcohol is 0.35. The lipoid content of the corpuscles should be a disadvantage. Warburg and Wiesel (175) studied the partition of various narcotics between erythrocytes and the saline in which they were living. Their figures were in many cases obtained indirectly but went to show that with methyl, ethyl, propyl and butyl alcohol, methyl

urethane and acetone there was less in the cells. In this series the amount in the cells increased but Warburg points out that he found the ratio cell to fluid less than 1 with butyl alcohol, while using the Overton Meyer $\frac{\text{oil}}{\text{water}}$ ratio it should be 6. He forgets that the cell is not all lipoid. He did not measure the lipoid content. While of methyl-phenyl-ketone twice, of phenyl urethane three times, and of thymol nine times as much were found in the cells as in the fluid. Again the striking statement of Nicloux and Yovanovitch (126) that the corpuscles contained 70 to 80 per cent of the ethylene to the plasmas 20 to 30 per cent is interesting in view of the relatively low adsorbability of such gases yet it does not furnish any real evidence in support of the Meyer theory. It should be realized that with the oil/water coefficient of the alcohols less than one the presence of the lipoids would be a disadvantage. But without lipoid analyses and accurate partition coefficients at the proper temperature such figures can be of no value as criticism or support. Usui (162), however, showed the absorption of thymol was little affected by extracting the lipoid from the corpuscles.

The distribution in the tissues of the body has again been examined by many workers but as a rule they have paid little attention to two considerations. First as Haggard (49) and Winterstein point out the rate of absorption of any tissue will be determined in part by the minute volume of its blood supply. That the brain shows a high quantity in the early stages of narcosis may simply be due to its large blood supply. Secondly, the amount held at any time depends entirely on the blood content and that this content must not have undergone any sudden change just before the organ is taken for analysis. For example, if an animal is killed at the end of a period with a large dose of chloroform, equilibrium to the amount present in the blood has not had time to occur. Thirdly, all observers seem to have assumed that the tissues will very rapidly come into equilibrium with the blood stream, that diffusion into the cell is very rapid. Of this there is no proof, indeed some of the figures suggest that this is not the case. A final equilibrium is only attained when the venous blood and the arterial blood have the same content. There is little or no evidence that this condition prevailed in almost all cases. Even Hansen (51), a recent worker, reports no analyses of arterial blood. We are indeed forced to rely on the length of time that the anesthesia had been maintained to judge whether the saturation is relatively complete or not.

Archangelsky (3) anesthetized rabbits with chloral, two cases, blood 0.143 and 0.156, brain 0.10 and 0.11, liver 0.026 and 0.019. In dogs his

figures are similar. His first case where respiration was failing at the end of fifty minutes shows blood 0.11, brain 0.039, liver 0.0047. Redonnet (134) analysed the organs of guinea pigs after giving neuronal and bromural, blood was always highest, the liver and brain practically identical. Leuze (85) anesthetized rabbits to loss of the corneal reflex only, with the volatile brom-containing anesthetics bromoform, ethyl bromide, prophyl bromide and dibromethylene. His average figures are given with the number of cases in brackets.

	Brain *mgm. per 100*	Liver *mgm. per 100*	Blood *mgm. per 100*
Bromoform (6)	0.0238	0.0129	0.0205
Ethyl bromide (2)	0.0715	0.0234	0.0319
Propyl bromide (2)	0.1005	0.0308	0.0529
Dibromethylene (2)	0.0136	0.0129	0.0089

Gensler (45) too worked with dogs and the brom derivatives, neuronal, bromural and adalin. On an average the blood contained 2 to 4 times as much as the brain and the brain about twice as much as other tissues. It may be noted that he found that with anesthetic doses of 0.25 gram neuronal per kilogram and with small non-anesthetic ones 0.1 gram the difference in the amount found in the brain was very small 28.1 to 20.3 mgm. per 100.

Nicloux and Yovanovitch (124).

DOGS, CHLOROFORM	I THRESHOLD DURATION 15	II DEFINITE ANESTHESIA FOR 1 HR.	III ANESTHESIA 1 HR. 30	IV ANESTHESIA FATAL DURATION 1 HR. 50
Cerebrum	26.6	46.2	42.8	52.5
Bulb	31.6	—	52.7	75.5
Cerebellum	20.7	—	—	55.8
Pneugastric nerve	73.7	71	114	166
Sciatic nerve	43.3	—	50	76.3
Arterial blood	28.2	36.3	48.1	56.7
Venous blood	—	—	44.8	50.8
Liver	22.8	35.8	37.9	46.1
Kidney	26.5	29.8	27.6	43.6
Spleen	18.2	—	19	22.2
Heart	20	—	—	40
Muscle	9.3	—	—	25.1
Lung	—	—	—	25.5
Fat:				
Skin	43.1	—	—	—
Omentum	38.7	—	—	—
Kidney	—	—	—	138

Nicloux (122–126) returned several times to this question. With Frisson (39) he showed that white cerebral tissue contained more than grey but based on lipoid (chloroform extractable material) slightly less.

Storm van Leeuwen (143) worked with cats and his figures have the advantage that there is a comparable degree of anesthesia throughout his experiments. The animals were given fixed narcotic concentrations with a respiratory pump.

Average figures in milligrams per 100

	BLOOD	SPINAL CORD	BRAIN ½	OTHER ½
Chloroform	0.032	0.038	0.0388	0.0385
Ether	0.105	0.111	0.108	0.105

In other experiments he used mixtures of ether and chloroform. Here too he found usually slightly more anesthetic in brain than in blood. In young dogs anesthetized to respiratory failure with ether and chloroform he found in average values:

	ARTERIAL	VENOUS BLOOD	BRAIN STEM	SPINAL CORD	HEART	KIDNEY
Chloroform	0.048	0.037	0.068	0.051	0.047	0.044
Ether	0.142	0.139	0.156	0.131	0.108	0.105

While the brain contains more than the blood the difference in relative anesthetic content of brain to blood with ether and chloroform shows no such striking difference as one would expect when the partition coefficients are according to K. H. Meyer 2.4 for ether and 70 for chloroform.

Hansen (51) has published a large series of analyses of the narcotic content of many tissues using as narcotics alcohol, acetone, ether and chloroform and has further analysed the tissues for ether extractable material, water and other solids. It is unfortunate that he gives very little evidence as to the stage of anesthesia reached by his animals and when he does mention it, the anesthesia was light and imperfect usually, but not necessarily of the same degree, save in the ether experiments. His animals were bled to death, the process in rabbits taking often fifteen minutes. Alcohol was given in some cases by injection, in others by inhalation, acetone by injection or by the stomach.

Neither of his animals which received chloroform subcutaneously were anesthetized. The cerebrum contained less narcotic than the

blood and less than liver, spleen, kidney. Fat had as much as ten times the blood. The degree of narcosis varying so greatly makes it impossible to compare the relative tissue content of the various anesthetics. He does call attention to the striking difference between the concentration of the various narcotics found in fat as compared with the blood. With alcohol the fat content was some 88 per cent less per hundred than blood, acetone 58 per cent less, ether in the two most comparable cases more by 80 and 95 per cent, chloroform more by 1096 and 1725 per cent. In one case where 7.322 grams acetone and 14 cc. chloroform were given the fat contained only $\frac{1}{3}$ the amount of acetone found in the blood but eleven times as much chloroform as in the blood. The quotient for acetone is 0.235 (Overton) and for chloroform 70 (K. H. Meyer).

His experiments with ether are more satisfactory. The concentration of ether was maintained approximately constant at a point such that the rabbits died of respiratory failure after a long time.

Rabbits anesthetized with ether to respiratory arrest

Quantities of ether expressed in parts per 1000

TISSUE	AVERAGE OF 5 EXPERIMENTS	DURATION OF INDIVIDUAL EXPERIMENTS IN MIN.					AVERAGE FIGURES PER CENT FOR		
		144	165	195	201	260	Ether extract	Water	Other solids
Venous blood	1.02	0.99	0.85	1.04	1.14	1.07	0.19	82.92	16.74
Cerebrum	1.12	1.15	0.98	1.27	1.12	1.06			
Cerebellum	1.20	1.17	—	1.29	1.22	1.10			
Corpora Quad.	1.45	—	—	1.45	—	—	9.19	78.16	12.50
Medulla	1.33	—	—	1.43	1.20	1.37			
Cord cervical	1.61	—	—	1.72	1.50	—			
Liver	0.99	1.04	0.69	1.11	1.09	1.03	4.01	74.10	21.79
Kidney	0.98	1.03	0.79	1.02	1.09	0.96	3.80	77.05	18.81
Spleen	0.80	0.61	0.56	1.09	—	0.95	3.19	76.61	20.45
Heart muscle	1.15	0.94	—	1.35	1.17	0.15	6.47	70.05	16.78
Skeletal muscle	0.75	0.62	0.54	0.92	0.86	0.82	1.67	75.39	22.85
Fat:									
Subcutaneous	3.58	1.94	1.42	4.75	4.44	5.33	90.38	7.31	2.06
Mesenterial									
Kidney	4.44	—	1.63	5.35	5.45	5.34	91.11	7.23	1.45
Preperitoneal	5.42	—	—	—	—	5.42	89.02	8.08	2.44
Urine	0.85	0.78	—	0.90	0.86	—	—	—	—

From these figures he calculates the degree of saturation of the organs and shows that the saturation in no organ was complete, brain being in

most cases most nearly saturated and fat least well. Unfortunately he uses the coefficient 4.5 for ether instead of the better figure 2.4 given by K. H. Meyer. When this is employed the saturation figures are much more in agreement. He further calculates the ether concentration in mols. per litre brain lipoid and obtains as an average figure 0.062 as compared with the K. H. Meyer figure (for a lighter anesthesia) 0.06.

In regard to this mass of evidence one must frankly admit that if one compares organs with large blood flow, brain, liver, heart, there is little evidence for a much greater excess in the brain as compared to the other two and this is particularly true of Hansen's figures for chloroform, if we take K. H. Meyer's figures for the partition coefficient as 70 and Hansen's figures for chloroform and average ether extract and water content as shown in the table below.

First chloroform experiment, Hansen

	CONTENT, PARTS PER L.	ETHER EXTRACT, PER CENT	WATER, PER CENT
Blood	0.30	0.19	82.92
Brain	0.30	9.19	78.16
Liver	0.34	4.01	74.10
Kidney	0.30	3.80	77.25
Heart	0.46	6.47	77.05
Kidney fat	3.66	91.11	7.23

These figures form the basis of a remarkable calculation by Winterstein (187), which the author has been unable to repeat. Winterstein's object is to show the futility of the application of the Meyer-Overton theory to distribution in the body. But the conditions of this experiment show that the primary postulate insisted on by Winterstein, namely, such a period of anesthesia that equilibrium has been reached was not present. The animals were not anesthetized and by an injection method no constancy of anesthetic condition can be obtained. Yet even taking such figures of those of Nicloux and Yovanovitch, experiment iv, where we find 52.5 in cerebrum, 46.1 in liver, and 56.7 in blood the differences are not great even between liver and brain. Cerebrum contains about 10 per cent lipoid, liver rarely more than 7 per cent and blood possibly 1 per cent. However, we do not know what the lipoid values in this case were, nor do we know the partition coefficient value at 38°C. In view of the peculiarity of the water solubility for chloroform at such a temperature, Landholt (77) (Chancel and Parmentier)

neither the Overton figure of 30, nor the K. H. Meyer figure of 70 based on lower temperatures are likely to apply. Further in the case of gaseous anesthetics K. H. Meyer has pointed out that the amount dissolved in the lipoid depends chiefly on solubility in lipoid at a given partial pressure rather than on the oil/water coefficient. Yet that solubility in fat does in part determine the distribution is evident when we consider the large amount found in fat or in nerves with their relatively poor blood supply. Further fat and nerves differ very radically in the character of their lipoids more so than nerves and brain. Meyer (108) in his review tacitly admits this criticism when he states that chemical analysis of the narcotic content of the tissues can neither upset nor confirm the theory. Possibly he would suggest that in the case of brain it is only the lipoids of the surface of the cells which dissolve the narcotic, though this does not agree with certain expressions of his views.

d. Hansteen Cranner (52) observed that plant rootlets give off lipoid material to water or salt solutions in which they were growing and then further he showed that the living cell walls of all parts of plants gave off either water-soluble or water-insoluble lipoids dependent on the temperature of the water in which they were placed. These lipoids were in large part phosphatides though they contained cholesterin also. These lipoids were denatured and became no longer water soluble when treated with high concentrations of lipoid solvents. While therefore agreeing with Overton that the essential constituent of the cell wall is lipoid supported perhaps by a cellulose framework, he disagrees with Overton's conception of the penetration of lipoid soluble constituents being due to lipoid solubility since he maintains that the physicochemical properties of cell lipoids are not those usually attributed to them as these conceptions have been derived from a study of the lipoids in their denatured state. He assumes that the water soluble lipoids can not be solvents for such substances as narcotics but that these and other substances enter the cell by being adsorbed on the lipoids which have a high capillary activity. Here he is in agreement with Loewe (95). In regard to the physical nature of non-denatured lipoids he is in agreement with Thudicum's study of cerebral lipoids. But surely a surface adsorption which may be a preliminary action can not explain the penetration of any normal cell constituent into the cell nor even of such foreign constituents as appear within the cell itself as some fat soluble dyes do, nor certain alkaloidal bases as shown by Overton. Further in his experiments he found that cloudy solutions of his lipoids became water clear on adding small amounts of ether. It may be that the ad-

sorption of ether decreased the degree of dispersion of the lipoid but his statements rather support the criticism of Meyer (108) that the lipoids were dissolved in the water containing ether in solution; that, in other words, they were ether soluble. Now as Meyer points out the small amounts of narcotics present in cells during anesthesia are far too small to produce a denaturation of the lipoid and may well be dissolved in the lipoid even though it shows some water solubility.

e. An objection that is, if accepted, fatal to the theory arises from the extension of the idea of narcosis to all forms of living tissue (Warburg, Meyerhof).

Höber and others as will be described in more detail at a later stage show that narcotics in a similar sequence of potency depress muscle activity and enzymatic processes whether intra- or extra-cellular and that this latter depression is produced whether lipoids are present or not. Meyer (108) admits the facts but considers that while narcotics present the same order of activity and reversibility, so that these inhibitions appear similar to those involved in anesthesia, nevertheless they differ in the quantities required and in the occurrence of oxidative depression. He further points out that the order of activity in other than homologous series has not been shown in all these cases to parallel their cerebral narcotic efficiency. In an homologous series an increase is to be expected as all physical properties change in a step-like fashion and if they are adsorbable this should also show increase. Meyer urges that such gases as nitrous oxide, methane and ethylene are not adsorbed by watery colloids and states that in personal experiments chloroform can not narcotize the catalytic combustion of sugar by charcoal. Meyer then attempts to show that solution in the lipoids not only forms the mechanism by which narcotics enter the cell but is the cause of the narcosis itself. He maintains that the presence of the narcotic in the lipoid "loosens" their connection with other cell constituents and considers that they increase the permeability of the cell wall and hence the ionic and other equilibria are disturbed.

VII. The adsorption theory, Warburg. As we have already seen in examining the precipitation theory certain evidence has been put forward that narcotics united with proteins and other, even inorganic substances in such a way as to lead to their precipitation. Such evidence we must review a little more carefully. The work of Lillie (86) which we have mentioned in connection with the asphyxial theory and which we will discuss again in connection with permeability changes had led

him to suggest that the narcotics were adsorbed on the surface and Traube too was led to consider adsorption as we have seen.

But in part contemporaneously with the above work a group of workers had been studying the mechanism of cellular oxidations and although in part their work served to overthrow the asphyxial theory in other respects, and with these we will now deal, they led to the formulation of a new theory.

Warburg (168) and his pupils were ardent workers in this field (81). Warburg (167) had studied the effect of narcotics on the respiration of the nucleated erythrocytes of the goose and shown that they depressed the oxidation of these and other cells.

In 1912 Warburg and Wiesel (175) published an important paper in which they summed up the previous work and extended it. It had been shown that narcotics of various types, ketones, methanes, nitriles, acid-amides and esters decreased the rate of oxidation of many kinds of living cells such as bacteria, yeast, fish spermatozoa, lymphocytes, liver cells, and red blood cells of birds: that the magnitude of the effect increased with concentration, was reversible and prompt (urethane in a given concentration had no greater effect in 100 minutes than in 15) and that the law of homologous series was followed. For example the rate of oxidation in Vibrio Metchnikoff was decreased 30 to 50 per cent by

		gram mols. per l.
Methyl urethane	5 per cent	0.67
Ethyl urethane	3.5 per cent	0.40
Propyl urethane	1.0 per cent	0.097
Isobutyl urethane	0.5 per cent	0.043
Phenyl urethane	0.05 per cent	0.003

The increase of yeast cells in the absence of oxygen was greatly decreased by methyl urethane 8 per cent, ethyl urethane 4 per cent, propyl urethane 2 per cent, and fermentation was likewise reduced.

Further they showed that the activity of the juice expressed from yeast cells was also depressed in a similar fashion by homologous series of narcotics and also that a precipitate was produced in such juice when the concentrations of the narcotics used were not much higher than those required to inhibit the oxidation of the yeast cells. The dried powder obtained by extracting yeast with acetone and ether and hence largely freed from lipoid, showed oxidative activity and this again was depressed by narcotics, for example, almost completely by ethyl urethane 6 per cent or 0.68 G.M. per L.

We have seen that Warburg and Wiesel's studies of the partition of narcotics between red cells and saline did not yield evidence of the effect of lipoid solubility. Usui carried the examination of thymol a stage further when he showed that the absorption occurred even with stromata of laked cells and was little affected even if the stromata had been extracted to remove lipoids. Here again we have evidence that the absorption was not due to solution in lipoids.

Battelli and Stern (6), 1913, studied the effect of narcotics on the oxidizing ferment, oxydone, obtained from various animal tissues and showed that narcotics reduced oxidation and caused precipitation. They again show a parallelism to the narcosis of polywogs though much higher concentrations were required. Vernon (164), 1914, criticized their conclusions experimentally. Warburg (169), 1913, studied the oxidation of macerated liver and found it took up oxygen giving off carbon dioxide but even after filtration of the macerate the fluid continued to act in the same manner but was not so active. He observed that the fluid under the microscope contained particles. Even after passing through a Berkefeld filter there was a little oxidation but not if the fluid was free from suspended particles. In 1914 (171) he showed that the oxidation in all these preparations was decreased by narcotics. Ethyl urethane depressed oxidation of the filtrate containing particles by 50 per cent when present in a concentration of 360 millimols per L. The Berkefeld filtrate required 720 millimols. Warburg (170), 1914, studied a respiration model of animal charcoal which adsorbs oxalic acid and leads to its oxidation. This too is inhibited by narcotics. The depression of oxidation is only in part due to the adsorption of narcotics on the charcoal driving off the oxalic acid. The adsorption isotherm has a lesser curvature than is evident in the case of bird's erythrocytes.

Similar models were studied by Meyerhof (110), 1914, namely, on invertase which was in part reversible, e.g., ethyl urethane 8 per cent or 0.90 mol. decreases the action of invertase 30 per cent and there was evidence of a change in the physical state of the enzyme by the narcotics. He could not produce evidence of a change in the degree of dispersion of the proteins by certain of the narcotics though he could with ether. Meyerhof (111) also studied the reaction between colloidal platinum and hydrogen peroxide which again was depressed by narcotics and was largely reversible and the narcotics did not appear to cause aggregation.

Loewe (95), 1913, published a long discussion of the action of narcotics in which he very strongly advocated the view that they were adsorbed and he is led to strongly urge the adsorption is due to the

lipoids. He, indeed, goes so far as to suggest that the figures published by Baum on the partition of narcotics between oil and water is due to adsorption in the oil and not to solution in oil. His arguments can hardly be accepted in view of the work of Wroth and Reid (190). This contention he supports by experiments on the conductivity of membranes containing cerebral lipoids and the effect of narcotics upon them. An examination of his curves, however, reveals that chloral acts in a sense the reverse of that of chloroform.

Meyerhof (112), 1916, studied the effect of narcotics on nitrifying bacteria; their oxidative processes were decreased, but again the adsorption curves were concave and not typically convex. In 1917, he showed the amount of narcotic required was small, a depression of 42 per cent being produced by 0.016 mol. of ethyl urethane. Chemical, as well as purely physical, factors appeared to be present. He (115) also studied the oxidative depression of acetone extracted yeast by narcotics.

Freundlich and Rona (36), 1917, studied the effect of narcotics on colloidal ferric hydroxide and showed that a narcotic increased greatly the precipitating effect of electrolytes present in the solution, since the adsorption of the capillary active narcotic on the surface of the colloid decreases the charge on the particle and consequently less electrolyte can neutralize the remaining charge.

Meyerhof worked with the juice from macerated yeast and showed that the clear ultra-filtrate showed the Tyndall phenomenon in the presence of narcotics in a concentration which caused precipitation in unfiltered yeast juice. This precipitation he also considers as due to the electrolytes present. The fermentation by yeast juice is also decreased as the substrate is displaced by the adsorbed narcotic. While with invertase and colloidial platinum proper adsorption isothermus were found, this is not the case with yeast juice as here the relationship is almost linear, in the case of nitrifying bacteria concave. Bierich (12) in view of Meyer's suggestion that narcotic values changed with temperature and also the work of Freundlich and Rona, Moore and Roaf, etc., on precipitation who all found greater effects at higher temperatures, studied the effect of temperature on the adsorption of narcotics by charcoal and found that there was little difference. The adsorption of isoamyl urethane, isobutyl urethane and heptyl alcohol was small. He also showed that the precipitation of ferric hydroxide and proteins by narcotics in the presence of electrolytes was aided by increase of temperature.

Warburg (173) in 1921 found that charcoal in water does not adsorb

gases as well as when dry. He showed that charcoal adsorbed cystine and also leucine and tyrosine from solutions and oxidized them. This process is depressed by narcotics. All this work then forced him to the theoretical statement of narcosis as follows (174).

If one adds to the same quantity of charcoal and of amino-acid in solution as much of different narcotics as displace an equal amount of the amino-acid and in the state of equal displacement measures the narcotic concentration in the solution c, and the quantity of narcotic adsorbed by equal quantities of charcoal, X, one finds the differences in the value of X as compared to those of C relatively small. For example, 1 gram charcoal had, when 0.03 milli-mol of cystine is displaced, adsorbed 0.6 to 1.5 milli-mols of various narcotics even though the concentrations of the various narcotics had differed by a thousandfold.

SUBSTANCE	C MOLS PER L SOLUTION	X MILLIMOLS PER G. CHARCOAL	$X(Vm)$ 2/3
Asymmetric dimethyl urea	0.03	1.1	9.0
Symmetric dimethyl urea	0.002	1.66	6.9
Phenyl urea	0.0002	0.76	8.7
Acetamide	0.17	1.2	7.3
Valeramide	0.003	0.62	6.9
Acetone	0.073	1.33	8.3
Methyl phenyl ketone	0.0004	0.73	8.0
Amyl alcohol	0.0015	0.87	7.9
Acetonitrile	0.2	1.5	7.7

He now makes the following assumption. The displacing substance (narcotic) takes possession of the surface of the charcoal in a monomolecular layer. The decrease of the surface so produced is the sole cause of the displacement of the adsorbed substance. Then the action will be independent of the chemical nature of the adsorbed narcotic and determined by the number, X, of the adsorbed molecules on the one hand and the surface covered by each molecule on the other. The conditions for this action are $XF=K$, where X equals the number of adsorbed molecules (this can be measured) and F the surface taken by such a molecule and equal to the area which the molecule conceived as a sphere takes up. If we designate the molecular volume obtained by refraction equivalents as Vm then F is proportional to (Vm) 2/3 or X (Vm) $2/3=K$. These values are shown in the last column, which indicates that these conditions are fulfilled with considerable accuracy.

According to Freundlich, there exists between the adsorbed quantity

X of a substance and its concentration in the solution the relationship $X = Kc\ 1/n$ when K and n are constants. If we replace the value of X in the former equation by this expression we have $Kc\ 1/n\ (Vm)\ 2/3 = K$.

From this equation we can calculate the effective strength of any narcotic, if K, the adsorption constant and the molecular volume are given.

Here as pointed out with enthusiasm by Winterstein we have a theory reduced to mathematical form and complete and pleasing in every ideal respect. Nor has the theory met with any criticism when applied to such models. However it is by no means possible to apply the same to ordinary anesthesia of the central nervous system. Nor is it perhaps justifiable to apply the theory to other model systems or to such simple types of narcosis such as that of birds erythrocytes where the adsorption isotherms are not of a normal convex type but linear. Other such examples are referred to above. These varying types of isotherms probably, however, simply mean that the means employed to obtain them do not measure simple adsorption but are measures of more than one type of change, not all of which may even be purely physical. Further recent studies of adsorption undoubtedly suggest that the molecules do not act in adsorption membranes as spheres but that such a substance as amyl alcohol, for example, stands off from the surface as an elongated molecule and that even more than a unimolecular layer can be formed (Langmuir 81). Such conceptions applied to the calculation in Warburg's table might give much less striking results.

Further it may be pointed out that all these models deal with enzymatic effects and chiefly with oxidation processes. Now we have abundantly shown that even in the lower forms of life, narcosis and depression of oxidations are not at all the same thing. Some of the evidence comes from Warburg himself, and doubtless he would admit that they are different processes requiring very different amounts of narcotics, yet he might reply that nevertheless the depression of other processes produced by adsorption, which have not as yet been studied may explain what we term narcosis.

IX. Permeability theories. Höber, Lillie and Winterstein. The theories of permeability are intimately bound up with the problem of stimulation and its accompanying effects, especially the changes of cell potential. These theories have thus far undergone various changes as the theories in regard to stimulation and changes in potential as shown by living cells have changed. As far as narcosis has been involved the question has been complicated by the fact that previously investi-

gators assumed that the absorption of a small amount of anesthetic caused stimulation while larger amounts caused depression. I doubt very much if this conception is as prevalent as in the past. Even in man there is much less evidence of stimulation with the newer anesthetic gases, possibly because the early phases are passed through as quickly, but in animals even in lower concentrations stimulation is not evident. We have also become better informed in regard to the reflex stimulation that occurs with many anesthetics and we have become more doubtful whether the so-called central stimulation effects are not due in a large part to the depression of the higher inhibitory cerebral control. The central stimulation does not appear with amytal given intravenously to man nor with hypnotics given to animals, nor is it evident with nitrous oxide or cyclopropane and propylene.

In 1907 Höber (59) published a paper in which he suggested that the adsorption of an anesthetic decreases the penetrability of the cell. This view he and his pupils have since supported. In 1909 Lillie (86) published the first of a series of papers in which he presented evidence that at the stage of narcotic depression of the movements of Arenicola larvae, there was evidence of a decrease of permeability. In a later paper (87) 1912, he showed that Arenicola larvae exposed to anesthetic concentrations of ether, chloroform, chloretone and alcohol did not undergo their characteristic contraction when placed in isotonic NaCl solution. The sodium chloride was prevented from entering and the loss of pigment accompanying the effect of the sodium chloride did not occur. Further narcotics decreased the permeability of Asterias' eggs after the artificial production of fertilization membranes (90). On Arbacia eggs (88) anesthetics prevented the effect of various salt solutions and in both cases prevented the exit of pigment from the cells. Lillie (92) considers that the permeability of a membrane is decreased by stimulation, that a change occurs in the ionic concentration of the membrane, hence that the presence of a narcotic adsorbed on the membrane decreases its permeability and hence prevents stimulation (92). These proofs of change of permeability were however indirect. More direct were Lillie's experiments (90) with sea urchin's eggs in which he measured the permeability for water by the change in their diameter. Here too there was a measure of not only the effect of narcotics in decreasing water permeability but also in their narcotic effect as judged by the inhibition of cleavage. Lepeschkin (83) initiated a new line of attack when he showed that the root hairs of Trianea when narcotized with ether absorbed less methylene blue and other dyes than when not

narcotized. Unfortunately repetition by others of his type of experiment has led to many contradictory results (Ruhland, 139; Collander, 24; see Höber, 61). Lepeschkin's experiments on the plasmolyses of the cells of Tradescantia produced by salt solutions and the inhibiting effect of narcotics upon this phenomenon has also been repeated. Lullies (99) working under Höber studied the effect of narcotics on the rate at which the plasmolysis of Tradescantia cells could be decreased by glycerine, etc., and the effect of narcotics on the process. He found that the entrance of glycerine and to a certain extent glycol could be decreased by narcotics in reversible concentrations but not that of urea. Osterhout (129) investigated the electrical conductivity of Laminaria and found again that narcotics in relatively low concentrations (ether 1 per cent) produced a reversible decrease of permeability. While Harvey (55) thought that the penetration of sodium hydroxide into Hipponoë eggs was increased by the presence of non-toxic doses of narcotics, he did not present evidence of the non-toxic character of the concentrations used and Loeb showed that narcotics delayed the shrinking of Fundulus eggs by hypertonic salt solutions but again we are not informed as to whether the concentrations may be regarded as narcotic ones. On red blood cells narcotics produce hemolysis in high concentrations but in low concentrations Joel, (67), Katz (68), Knaffl-Lenz (71), Jarich (64) have shown that hemolysis by certain other agents is decreased. Siebeck (141a) measured the rate at which erythrocytes lost chloride in isotonic sulphate and sugar solutions and found that the presence of low concentrations of urethanes decreased the rate of loss but not the final amount. McClendon (104) showed that low concentrations of narcotics hindered the loss of chloride from the eggs of Esox in nitrate solutions. Higher concentrations here as in the case of many experiments by other workers increased the rate of loss.

Winterstein (184, 186) studied the influence of narcotics on membranes made from the thin belly muscles of female frogs. Small disks of muscle were bound over the end of a glass cylinder filled with normal salt solution. This end of the cylinder was placed in unphysiological solutions and the osmotic changes produced and the transfer of chlorides through the membrane could be followed. Narcotics in concentrations which were truly narcotic for the muscle, decreased the permeability of the membrane for water. Toxic concentrations increased the permeability. One difficulty with these experiments is the possibility of the change in permeability taking place in part in the connective rather than the muscle tissue, which Schulze attempted to demonstrate and also to the

fact that though the muscle was in contact with a normal solution on the one side the other was exposed to far from normal conditions. This is also true of Arani's (189, p. 386) experiments in which isotonic solutions of nitrates or sulphates were placed on the far side of the membrane. Winterstein also observed that after the removal of the narcotic the membrane showed an abnormally increased permeability.

Embden (34) noted that frog's muscle in isotonic cane-sugar solutions loses phosphate. The same occurred in solutions of narcotics. His pupils, Lange and Möller (79) showed that, as judged by the loss of phosphates, the muscle in reversible narcotic solutions of low concentrations showed a decreased permeability but in higher concentrations though still reversible the opposite was seen and Lange and Kappus (78) conclude that muscle narcosis is due to an alteration of the muscle cell membrane which may be evident as an increase or decrease of permeability, but that the essential change consists in an inability of the membrane in narcosis to react to such influences as would normally change its permeability.

Gellhorn and Weidling (46) using as the criterion of permeability the change in hydrogen ion concentration in a solution surrounding the muscle found that narcotics produced a decreased permeability. Wertheimer (177) and others have studied the effect of narcotics on the penetrability of the frog's skin (or human skin) as judged by potential changes or dyes with in part conflicting results, though the balance of evidence is in favour of decreased permeability. As we have however no proof of narcosis occurring in the membrane they will not be further discussed.

Winterstein (189, p. 393 et seq.) then considers that these experiments show that narcotics decrease the permeability of the cell membranes. The adsorption of the narcotic decreases the available area for the transmission of water or salts, nor is there any need to postulate that the adsorption occurs predominately or exclusively by the lipoids. In higher concentrations it is true that the narcotics may dissolve out cell lipoid and hence produce the irreversible increase in permeability which so often is seen under these conditions. But even here this explanation is not necessary for we have seen that high concentrations of narcotics cause the coagulation, or further the coagulation of non-lipoid bodies and that this or the decrease of swelling observed by Kochmann and others may amply account for the increase in permeability. Thus we see that Höber, Lillie and Winterstein would attribute narcosis to a decrease in cell permeability. Winterstein certainly considers of little moment

the observations which are opposed to this view but the others while emphasizing the parallelism are more interested in the change in the membrane itself, though considering that this is usually expressed and in a sense can be measured by a decrease in permeability. Lillie, however, lays a certain stress on the change in the electrical state of the membrane produced by the narcotic. He (92, p. 207, 216) suggests, indeed, that the narcotic adsorbed on and possibly later dissolved in the membrane introduces such a modification that the membrane is in a physically more stable state. Here he agrees with the conception of Lange and Kappus. Once such a change is produced the cell is narcotized and, if I understand him aright, no penetration of ions, salts or water is required. The theory then becomes one of change in the physical state of the membrane accompanied, it is true, by a change in electrical condition.

Winterstein tends to deny the importance of the lipoid structure of the membrane, Lillie and Höber seem convinced that the lipoid materials are the important elements concerned.

X. Beutner's theory. Jacques Loeb and Beutner (93), 1913, were able to show that reversible changes in potential occurred in a model step system of the gas chain type which consisted of

calomel electrode	apple or leaf	M/X KCl solution, calomel electrode

when the KCl concentration was altered. Further they simplified their model by using in place of the apple or leaf lecithin dissolved in guaiacol. With these models they showed that various narcotics, methyl, ethyl and propyl alcohols when added in relatively high concentrations to the solutions changed the potential differences in the system. These changes were more marked with the apple than the lecithin model. Ether and chloral caused similar changes.

To these studies Beutner (10) 1927–29, has recently returned. His experiments both with models and with frog's skin suggest the importance of lipoid. Starting from the theory that stimulation of tissues is due to a disturbance of the existing electrical potentials, stimulation occurring when the polarization of a membrane reaches a definite value, Beutner assumed that when an alkaloid, pilocarpine, produced stimulation a similar change in potential would occur. He then set up a gas chain model

– Hg	Saturated KCl Soln.	Nitro benzene 10 per cent oleic acid	physiol. NaCl 0.02 Na oleate	KCl saturated with HgCl	Hg

and when pilocarpine in therapeutic concentrations was added to the sodium chloride solution the potential difference fell. The same was true with other alkaloids. In this model narcotics produced no change in electromotive force.

From these studies Beutner and Hyden returned to a collateral study which Beutner has for some time been following. He and others have shown that certain alkaloids added to serum became absorbed "in a way similar to adsorption" and then failed to diffuse through a parchment membrane. If, however, narcotics, especially alcohols, were added more of the alkaloid diffused out. In the homologous series of alcohols there is an increasing effect in the lower members but above $C_6H_{13}OH$ the effect decreases again while the narcotic effect increases. Here, he suggests evidence that narcosis is not due to adsorption on proteins but that preference should be given to a theory based on lipoid solubility or to a theory of change in potential due to the effect of narcotics on lipoids, such as would arise out of Beutner's previous work. This may be summed up in the conception that the effect of narcotics on a lipoid containing membrane is to make such changes as become evident when the previous with the new potential differences on either side of the membrane are compared.

CONCLUSION

We have seen that certain of the theories of narcosis are no longer possible. Three well formulated theories have been presented, namely, the lipoid theory, Meyer-Overton, the adsorption theory of Warburg and the permeability theory of Lillie and Winterstein, if the latter can be called a distinct theory. The Meyer-Overton theory is not disproved as yet, the parallelism between narcotic power and partition coefficient the calculations of K. H. Meyer on the constancy of the gram-moleular content of the brain lipoids as yet stand. The effect of changes of temperature upon the narcotic potency and partition coefficient has received as yet more valid support, than adverse experimental criticism. The evidence in regard to the effect of temperature changes on adsorption at present all goes to show that the adsorption of narcotics does not change in different directions with temperature with different narcotics. Yet this evidence in regard to Meyer's theory has been most strongly attacked and but for the fact of the possibility that the rapidity of the temperature changes of Meyer's supporters as well as his opponents may have introduced such changes in metabolism in many cases as to vitiate the results this support might have crumbled under the attack. It

presents nevertheless a satisfying picture for the practical anesthetist as it explains the important progressive storage of an anesthetic during the course of an anesthesia due in part to the absorption of the anesthetic by the fats in the body. The remarkable amounts of anesthetic found in the fatty tissue in the experiments of Nicloux, Nicloux and Yovanovitch and Hansen can hardly be explained in any other way. But the figures on the distribution of narcotic in the body tend to suggest that possibly the adsorption, which we are forced to consider a possible preliminary step may account for the anesthesia of the central nervous system.

Warburg's beautifully expressed theory of adsorption is, however, on closer observation even less satisfying. Adsorption on what and any type of colloid, lipoid or protein, on the cell surface or particles within? More fundamentally still, how can it ever be proved when there is no clear definition or measure of adsorption? Our only measure of whether adsorption has occurred and not solution or chemical action is a study of the adsorption isotherm, the change in the amount adsorbed with change in concentration in the medium with which the colloid surfaces are bathed. We have, however, seen that adsorption isotherms have been presented by the author of this theory and by numerous colleagues which are not at all typical isotherms, even the reverse of typical or linear. The method of measuring the change may be at fault, i.e., Meyerhof when working with nitrifying bacteria judged the adsorption by its result on the bacterial metabolism. Other factors than mere adsorption may play a part, such as chemical changes or the adsorption may be but a preliminary step to solution. In view of all the evidence we can not believe that the lipoids are not essentially involved in the action of the narcotics. Even such opponents as Traube have been driven to this conviction. Loewe, who stands for adsorption, considers the lipoids the essential substrate on which narcotics act. Winterstein even admits that they may play some part. The facts of Hansteen Cranner really support the preëminent importance of the lipoids in the necessary daily life of the cell and may be well considered as proving their universal distribution, their labile character and even their suitability as a point of attack of narcotics, though even he would deny that the narcotics affect the cell by being dissolved in their lipoid coat. Our knowledge of physical chemistry and especially of the lipoid colloids is still too vague and subject to change to allow us to make a mental picture or theory based on adsorption alone on the cell lipoids. Höber distinctly recognises the difficulty both in his book and in his

review from which I may be permitted to quote (p. 436). He has been reviewing the question of the penetrability of the cell, "We end again with a lipoid theory of the surface of the cell, only that in this consideration lipoid has been regarded not as a solvent but as a substrate for adsorption. Between these two views (solution and adsorption) it is however by no means easy to decide. In the meanwhile there is still a lack in these as in other cases of the necessary proof of the adsorption theory; the effect on the surface tension between water and protoplasm has been even less investigated than the partition between water and lipoid."

Further no theory of anesthesia will prove acceptable which is based on a proof of a depression of the resting oxidation of the cell. It may be a narcosis but it is not the narcosis of reflex movement which we may more narrowly call anesthesia. Nor will a theory of adsorption explain the large amount of anesthetic in fat depots. Surely fat cells are more homogeneous and present fewer colloid particles or surfaces than any tissue we know.

Nor is the theory of adsorption necessarily compatible with the theory of permeability. Presumably neither the narcotic nor the sugar penetrate Warburg's charcoal particles, though it might be suggested that the narcotic blocks the pores in the particles and prevents the sugar entering, but the anesthetic must also enter and push the sugar out. This is not, I think, the process which is suggested when it is said that an adsorbed narcotic decreases the permeability of the cell. But before we accept this theory is it fair to ask what substances are prevented by the narcotic from entering, certainly not oxygen. Then is it ions? Is it not much simpler to think with Freundlich and Rona, for example, that the adsorbed narcotic decreases the surface charge available for the production of potential differences, that there is a stabilization as suggested by Lillie? There is surely no need of a permeability change and though we have presented evidence of a change in permeability to certain tests, these have in most cases imposed abnormal strains upon the cell and even with such tests the evidence is not harmonious as may be well seen by reading such studies as those of Höber and Osterhout.

We have repeatedly seen that our theories of narcosis are patterned on the changes produced by narcotics in relatively simple models, oil/water partition, adsorption on colloids, membrane changes, etc. Unfortunately we are only able to think in such simplified terms and in reality our theories are simply descriptions of processes. As such the Meyer theory seems to me to most nearly fit the needs of the anesthetists

and the adsorption theory of Warburg to be the simplest and probably far too simple. This, indeed, would be my criticism of the Meyer theory also. Meyer admits adsorption as a preliminary phase. I would be inclined to think that the evidence suggests that it is an important phase in the case of the central nervous system but solution in fats and watery constituents elsewhere also plays a part in the absorption of anesthetics over the long periods of time of many anesthesias.

The final chapter in the theories of narcosis has as yet to be written. The author feels that neither adsorption nor solution can explain the peculiar inherent toxic properties of a stable gas like butylene as compared with propylene nor the different effects of just narcotic concentrations of different anesthetics, for example, chloroform and ether or chloral and isoamylethylbarbituric acid on blood pressure.

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THE ABSORPTION, DISTRIBUTION AND EXCRETION OF BISMUTH

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A great number of clinical data and observations on bismuth have been published since the time of its introduction as an antisyphilitic. The clinical application of the bismuth treatment has been discussed recently by H. Mueller (107) in a review in the Handbuch der Haut- und Geschlechtskrankheiten. In addition to its efficiency in the treatment of syphilis, many clinicians, as for instance Brown, Salleeby and Shamberg (17), consider bismuth superior to mercury, because it also has the advantage of being less liable to give rise, clinically, to toxic effects, mainly because there is a greater latitude between the therapeutic and the toxic dose. This holds true especially for the intramuscular injections and for oral administration, but severe toxic symptoms may be produced with intravenous injections. F. Juliusberg (53) gives an extensive review on the side actions of bismuth in the treatment of syphilis, and R. Gottlieb's warning that no bismuth compound is absolutely harmless, may be cited.

After the appearance of the first publications on the use of bismuth against syphilis by Sazerac and Levaditi (121–124), numerous bismuth preparations have been advocated for clinical use, but the question, as to which type of bismuth preparation is the most valuable therapeutically, is still open for discussion; i.e., whether soluble or insoluble preparations are to be preferred. Since the question can be elucidated only by comparing the clinical efficiency with the absorption, distribution and excretion of different bismuth preparations, the study of these factors has always been considered to be of importance. Lomholt (94) recently has published a detailed review on experimental studies of bismuth; but as the scope of the present paper is confined to studies on absorption, distribution and excretion of bismuth in the human and in the animal organism, and to the discussion and reconciliation of different opinions, the present review may be helpful for further experimental investigations along this line.

CHEMICAL METHODS

In studying the absorption, distribution and excretion of bismuth, fractions of milligrams are considerable amounts, and the classical methods of quantitative analysis are therefore not sensitive enough for this purpose. This, and the necessity of digesting rather large quantities of organic material previous to the analysis proper, has stimulated the development of special chemical methods which will be discussed in the following.

I. METHODS FOR THE DIGESTION OF ORGANIC MATERIAL. For the destruction of organic material some investigators, as Autenrieth (5) and Akamatsu (1) have used the Fresenius-Babo method of digesting the material with potassium chlorate and hydrochloric acid. This procedure, however, leaves considerable quantities of ashes, for the solution of which comparatively large amounts of water are required. Its application is therefore limited to those cases where either small amounts of organic material are to be digested, as for instance, in urines; or to larger quantities of bismuth which permit a precipitation from the aqueous solution either by means of the electric current or by chemical reagents as hydrogen sulphide, alone, or in the presence of some other metals not interfering with the further determination.

The same limitations hold true for the digestion used by L. Grumach (48) with alkali, followed by neutralization and final destruction with nitric acid.

In all cases where large amounts of organic material are to be digested, or where only minute quantities of bismuth are present, digestion with acid is preferable, because this leaves the smallest amount of ashes. The digestion with sulphuric and nitric acid as used by Girard and Fourneau (42), Malengreau and Delrue (99), Leonard (71) and Hanzlik (101) holds the danger that with continued heating some insoluble bismuth compound, presumably bismuthyl pyrosulphate, is formed, thus producing a loss of material

Therefore, the digestion with nitric acid alone, or with hydrochloric acid and hydrogen peroxide as employed by Caille and Viel (19), Christiansen, Hevesy and Lomholt (25) and Sultzaberger (130) seems to be preferable.

In digesting the material with oxidizing agents, care must be taken that no oxidizing material is left when the digestion is completed, as this might interfere with the further determination. Therefore, the solution of the extract of the ashes should be tested with potassium iodide starch

paper; and in case of the presence of oxidizing material, the evaporation with acidulated water should be repeated until the last trace has been removed. According to Rasmussen, Jackerot and Schou (120), the destruction of oxidizing agents can also be accomplished by the addition of a saturated solution of oxalic acid. It seems, however, that with this procedure an excess of oxalic acid should be avoided, because excessive amounts of oxalic acid produce a loss of small quantities of bismuth, which indicates that this method requires further study.

II. Analytical methods. The analytical methods used for the determination of bismuth may be grouped into gravimetric, electrolytic, radioscopic, spectro-photometric, and colorimetric methods.

The gravimetric methods of ordinary quantitative analysis can be applied only to determinations in which large quantities of bismuth may be expected and they require large amounts of material. The micro analytical method described by R. Strebinger and W. Zins (129) and used by Weitgasser (132) requires special apparatus and thorough training in this excellent but tedious method, and its use is therefore limited.

In the electrolytic determination, the bismuth is precipitated on a platinum electrode, the increase of weight of which is then determined gravimetrically. Kuerthy and Mueller (61) used as anode a platinum dish of 50 cc. capacity and a rotating platinum cathode of 2.5 cm^2 surface with 800 to 1000 revolutions per minute. The electrolysis is made at 80–90°C., using a current from a small storage battery with a rheostat interposed. Statements as to the current used are not given; the authors emphasized the importance of the raised and uniform temperature. The electrolysis was finished at the end of half an hour and the electrode was weighed on a micro balance. L. Grumach (48) also used an electrolytic method, developed by Eisenlohr, with a similar arrangement and a current of 0.5 to 1 amp. A. Marcozzi (96) used a rotating cathode, consisting of a platinum screen of 4 volt tension and 950 ohm resistance corresponding to an initial current of 0.13 amp. Masson (97) employed two platinum gauze electrodes of 10 square centimeters and performed the electrolysis at 45 to 50°C. with a current of 2 volts for from eight to ten hours. The electrolytic methods require the use of micro balances for the range of quantities of bismuth which are found in tissues or excreted in the urine.

Christiansen, Hevesy and Lomholt (25) developed a radioscopic method. They added to the bismuth preparation a hydrochloric acid solution of the isotopic radium E, derived from a quantity of radium

of 2 to 4 curies. They injected this material and compared the radioactivity of the ashes of the different specimens with that of the original mixture. This method requires special apparatus; furthermore the duration of the experiments is limited to from twenty to twenty-five days, depending on the amount of radium E used. In our own experience, it seems also that with smaller quantities of the isotopic bismuth, the results depend, to a certain extent, upon the distribution of the ashes during the final electroscopical determination.

Rasmussen, Jackerot and Schou (120) determined the concentration of a bismuth potassium iodide solution, prepared according to the directions of Leonard, by means of the spectro-photometer, and in this way checked the exactness of the colorimetric method of the latter. A similar procedure was used by L. Lafay and F. Ferrand (65).

Colorimetric methods for the determination of bismuth are numerous; this indicates that it is not easy to work out a reliable procedure. These methods may be divided into two groups; the first employs the colored solutions of complex or double salts; the second matches the color of a colloidal solution against a standard. For a number of colorimetric methods, bismuth iodo-quinine solutions are utilized. Léger (69) used as reagent, a solution of 1 gram of cinchonine and 2 grams of potassium iodide in 100 cc. of water, slightly acidulated with a small quantity of nitric acid. With bismuth this reagent forms an orange precipitate in dilutions down to 1 to 500,000. This precipitate is soluble in alcohol and may be used for colorimetric comparison. It is important, however, that the solution is free from sulphuric and hydrochloric acid.

This method was modified by P. Aubry (3), who used two solutions, one containing 1 gram quinine sulphate in 10 cc. of water with the addition of 3 to 4 drops of sulphuric acid; the other containing 2 grams of potassium iodide in 10 cc. of water. Both solutions are mixed and diluted to 100 cc. with water. The ashes of the material which was digested with nitric acid and evaporated to dryness are dissolved with small quantities of water acidulated with nitric acid. Two cubic centimeters of the reagent are mixed with one-half cubic centimeter of the bismuth solution. In the presence of bismuth, a yellow color or an orange precipitate is formed. The sensitivity of this method is 1:600,000; but since iron forms very similar precipitates, the results may be misleading.

C. E. Laporte (67) used a similar procedure; but dissolved the precipitate in acetone, and used this solution for colorimetric comparison. Girard and Fourneau (42) found that bismuth in the presence of potas-

sium iodide forms a red precipitate with a benzene solution of tetracyl ammonium hydroxide, which is soluble in this solvent, and which can be used for colorimetric comparison. For this determination, the organic material is digested with sulphuric and nitric acid, and the final residue is dissolved in a solution containing 200 grams of sodium formate, 30 grams potassium iodide, 5 grams sodium sulfite, and 5 cc. of formic acid per liter. This solution is said to prevent the liberation of iodine by iron.

Léger (69), Laporte (67), and also Girard and Fourneau (42) used very volatile solvents for their colorimetric determinations, which involves the danger of evaporation of the solvents, and consequently of too high readings.

P. W. Dankwortt and E. Pfau (31) after digestion with nitric acid, added a solution of cadmium sulphate, precipitated with hydrogen sulphide, dissolved the precipitate in nitric acid, and determined in this concentrated solution the bismuth according to P. Aubry's method. One five-thousandth milligram may be detected with this method. Iron and copper are said to interfere with this determination.

C. H. Hill (50) acidulated the bismuth-containing solution slightly with nitric acid, and added to 50 cc. of this solution 0.3 gram of urea and 0.5 gram of antipyrin. While dissolving these by a thorough shaking, small crystals of potassium iodide were added until a red color was produced. This color was then matched against a solution containing 0.62 gram of bismuth subcarbonate, and 10 cc. of nitric acid per liter.

Arppe (2) described a potassium bismuth iodide, which is souble in acidulated water, producing a greenish yellow color. This compound was used by Laubach (68), Autenrieth and Mayer (5), and by Levy and Selter (87) for the colorimetric determination of bismuth. The digested material was dissolved in water, acidulated with hydrochloric acid, and 10 to 20 drops of 25 per cent solution of potassium iodide were added. After removing by means of chloroform the free iodine, which was liberated by the oxidizing agents, the color was matched against a standard solution.

Leonard (71) and Sultzaberger (130) used the same procedure, but instead of extracting the liberated iodine with chloroform, they prevented its liberation, and reduced the small quantities formed by the addition of sulphur dioxide water or by a solution of sodium bisulphite. Sei and Weise (127) found, however, that sulphurdioxide water itself is apt to produce a yellow color; to avoid this, they used stannous chloride solution in sulphuric acid.

Leonard (71) and Mehrtens, Hanzlik, Marshall and Brown (101) used a bismuth solution acidulated with sulphuric acid, which gives a somewhat darker color than that containing hydrochloric acid as used by Sultzaberger.

Recently F. de Myttenaere (114) described a method, advocated by Bouillene and Dumont: The material was first digested with nitric and sulphuric acid, evaporated to dryness, incinerated, and the ashes dissolved in a little nitric acid, and filled up to a fixed volume with water. Measured quantities of this solution were added to 10 cc. of water, and then 3 to 4 drops of 10 per cent potassium iodide solution and 2 drops of 1 per cent sodium bisulphite solution. The yellow color appearing within five minutes was compared with the color of a series of standards.

Iron, lead, copper and arsenic are said to interfere with these determinations. If these metals are present, they must be separated with the ordinary analytical methods; if only small amounts of iron are present, according to Leonard, it may be sufficient to add 5 cc. of a 10 per cent solution of sodium citrate, in order to reduce the ionization of the iron salts.

One of the methods developed by Kuerthy and H. Mueller (60) is based on the precipitation of bismuth with known amounts of ammonium phosphate, followed by the determination of the excess of phosphate by the Bell-Doisy method, transforming the phosphate into phosphomolybdic acid by the addition of molybdic acid. The molybdic acid is then reduced by hydro quinone in alkaline solution to molybdenoxide, the blue color of which is matched against a standard. The procedure seems to be rather tedious; the authors give control determinations down to 0.1 mgm. of bismuth.

There remain to be discussed several methods in which a colloidal solution or suspension is matched against a standard.

Kuerthy and Müller (61) acidulated the bismuth solution with nitric acid and diluted it so that it contained from 0.5 to 2.0 mgm. of bismuth per 10 cc. This solution was mixed with 10 cc. of a 1 per cent solution of acacia and 5 cc. of a saturated solution of hydrogen sulphide. Colloidal bismuth sulphide was formed which was matched against a standard solution.

W. Engelhardt (36) concentrated the bismuth after the digestion by adding zinc chloride and precipitating both metals with hydrogen sulphide. In the solution of the sulphides the bismuth was determined as colloidal bismuth sulphide.

F. Malengreau and G. Delrue (99) digested with nitric acid and sulphuric acid, and added a few cubic centimeters of a concentrated solution of ammonium chloride, and neutralized by means of concentrated ammonia. In this way, bismuth oxychloride, ammonium magnesium phosphate and ferrous hydroxide are precipitated. The precipitate was dissolved in hydrochloric acid and diluted to 10 to 20 cc. To this solution, 1 cc. of a 1 per cent solution of gelatine was added, and the metal was precipitated with hydrogen sulphide as colloidal bismuth sulphide. Two thousandth milligram of bismuth is said to be enough to give a distinct yellow color.

Sei (126) dissolved the digested material in 20 cc. of a mixture containing 20 cc. of 10 per cent tartaric acid solution, 20 cc. of 10 per cent sodium hydroxide, and water to 200 cc. To this solution 5 cc. of a 20 per cent solution of sodium sulphide were added, and this mixture was thoroughly shaken. The resulting black color was matched against a standard prepared in the same way, containing 0.2 mgm. of bismuth.

Cuny and Poirot (29) modified Aubry's method and prepared an aqueous colloidal solution by performing this reaction in a solution of acacia. The optimal concentration for this reaction is 0.25 mgm. of bismuth in 5 cc. Silver, mercury, lead, antimony, copper and iron are said to interfere with this determination.

Although these methods, in which colloidal solutions or suspensions are used for comparison, may give uniform results in the hands of their authors, the size of the submicrons which is closely connected with the color, depends on so many factors which are difficult to control, that their general use seems to be limited.

THE ABSORPTION OF BISMUTH FROM THE SITE OF THE INJECTION

Clinically, intramuscular injections are generally used; therefore, the following discussion will be restricted to the absorption of bismuth after intramuscular administration. The absorption of bismuth from the site of the injection may be studied in three ways, either by studying histologically the change of the bismuth deposit, or by following the change of the x-ray picture, and by chemical determination of the bismuth at the site of the injection.

The process of the absorption of bismuth from the site of the injection is still under discussion and it is difficult to say a priori whether soluble or insoluble preparations are more irritant, since the former may be precipitated at the site of the injection, and the latter may form soluble compounds. Levaditi and Nicolau (80) assumed that the bismuth forms a compound with the proteins of the organism by complicated chemical

reactions, the nature of which is unknown, and that it circulates in the organism in this form. They believe that this compound is formed by both soluble and insoluble preparations, the speed of the reaction depending upon the physical and chemical properties of the preparation. On the other hand, some of our own observations indicate that compounds of a similar type, that is, sodium bismuth citrate and sodium bismuth tartrate may behave quite differently at the site of the injection. After the injection of aqueous solutions of the same bismuth content of sodium bismuth citrate (2.5 per cent) and sodium bismuth tartrate (2.1 per cent) into the anterior chamber of the rabbit's eye, we observed that the tartrate immediately formed a precipitate, with more or less marked symptoms of inflammation which disappeared within seven days on an average; with sodium bismuth citrate, there was no immediate reaction, but within the following days a precipitate was formed with more or less marked inflammation lasting for about 10 to 15 days.

I. HISTOLOGICAL STUDIES OF THE SITE OF THE INJECTION. Since the absorption of drugs from the site of the injection is largely influenced by the reaction of the tissue, i.e., inflammatory processes, histological studies of the site of the injection have been made by several investigators.

A. Histological methods for the study of the localization of bismuth in the tissue. The simplest method for the detection of bismuth in the tissues is to form the black bismuth sulphide by fumigation with hydrogen sulphide or by staining the sections in saturated solution of this gas. This procedure was used by von Oettingen, Todd and Sollmann (115), by Cristeller (28), and by Ginji Komaya (58). The latter stained the tissues afterwards with lithion carmin. With this procedure the bismuth pigmentation is stable, but other heavy metals such as mercury may give similar dark deposits, so that the reaction is not specific.

Levaditi, Nicolau, Schoen, Girard and Mannin (82) after fixation in Dubosq-Brasil-Bonin solution for four to five hours and rinsing in water for six hours, stained the bismuth deposit for six to eight hours in a solution containing 94 parts of water, 4 parts of potassium iodide, 2 parts of quinine sulphate, followed by rinsing in distilled water and treatment with alcohol, xylene and paraffine. The paraffine having been removed, the sections are kept in a solution containing 96 parts of water, and 2 parts each of potassium iodide and quinine sulphate. A similar method was used by Ginji Komaya (58). The disadvantage of these methods is that the staining of the bismuth deposits is rather unstable and that it is easily decolorized by alkali.

B. Histological studies of the reaction of the tissue after the intramuscular administration of bismuth. One of the earliest reports on histological studies at the site of the injection is that of H. Mueller, Blass and Kratzeisen (110). After intramuscular injection of 0.05 gram Nadisan, corresponding to 0.005 gram bismuth tartrate in a two months old child, they found the following picture in the first three or four days: The area adjoining the bismuth deposits seemed to be necrobiotic, this zone was surrounded by cellular infiltration, with lymphoid and plasma cells outside of the blood vessels. There was also a proliferation of the perimysium, and single polymorphic leucocytes. In another case they also observed proliferation of the interstitial tissue and beginning granulation tissues. The whole area of reaction surrounding the bismuth deposits had a depth of approximately one millimeter.

Barthélémy (9) (10) and Gougerot (44) also reported on local tissue lesions produced in man after the injection of bismuth preparations, and P. Aubry (4) gave the analysis of the deposit found in an abscess, formed after the injection of an oily suspension of bismuth hydroxide.

Since such studies in man can only be performed with necropsy material, their number is limited, and systematic studies have been made on animals; by H. Mueller and Blass (109) (111) on rats and rabbits; by R. Jaffé (51) on rabbits; by Levaditi and his co-workers (82) on rabbits; by L. Grumach (48) on mice; and by Gruhzit (47) on rabbits, guinea pigs and dogs.

R. Jaffé (51) distinguishes two groups of tissue reactions, those which show only necrobiotic reactions, and those which consistently show formation of abscesses. Since the preparations used are only given with the code number of the institute, they do not permit any conclusions.

O. M. Gruhzit (47) distinguishes four groups of preparations: 1, water-soluble, but tissue insoluble preparations; 2, water-soluble and tissue-soluble preparations; 3, insoluble salts in oil or water suspensions; and finally 4, metallic bismuth in water or in oil suspension.

The water-soluble, but tissue insoluble preparations form precipitates with the tissue or with the tissue juices. To this group belong the bismuth tartrates and citrates. These preparations spread along the fasciculi of the muscle bundles, where they are precipitated. The tissue in contact with these deposits becomes necrotic, the intensity of this necrosis varying with different preparations. Later on this necrotic area is surrounded by a zone of fibrosis, and a slight infiltration with leucocytes is observed. After ten days, a capsule of connective tissue is formed, the necrotic material within which is slowly dissolved, and it takes weeks until the scar formation is finished.

The water and tissue soluble preparations, as bismuth thioglycolate, also spread along the fasciculi, but since they are not precipitated, they are rather rapidly absorbed, and, therefore, produce very little or no local irritation. Though there is some local edema, there is no leucocytosis, and the necrotic tissue gradually passes into a quiescent scar.

With the injection of insoluble preparations in water or in oil suspensions, the histological changes seem to be more dependent upon the chemical constitution of the preparation, as already indicated by the results of R. Jaffé. By the injection of such preparations which represent more or less viscous suspensions, considerable local trauma may be produced mechanically by destruction of muscle fibers, blood vessels and nerve fibers. With these preparations, the necrotic processes are therefore usually more pronounced, the incapsulation of the necrotic area is more marked, and abscess formation may occur. Usually it requires many weeks until the scar formation is completed. It appears that the destruction of the tissue is more marked with aqueous than with oily suspensions.

The injection of metallic bismuth in watery suspension seem to produce less immediate injury than the suspensions of insoluble salts. But after some time, necrotic processes develop also, together with symptoms of regeneration, and a progressing fibrous incapsulation of the metallic deposits, which still show extensive lime deposits and caseous masses after a period from seven to eight weeks. This calcification may be observed with many preparations especially in rabbits. von Oettingen, Todd and Sollmann (115) described the formation of insoluble bismuth soaps after the injection of watery suspensions of bismuth hydroxide. That such soap formation may also be formed in man is shown by the report of P. Aubry (4) mentioned above.

C. Histological studies on the disappearance of bismuth from the site of the injection. The fate of bismuth at the site of the injection varies very much with the type of the preparation. According to experiments of Levaditi and his co-workers (82) with water soluble but tissue insoluble preparations, such as sodium potassium bismuth tartrate, there was an impregnation of the muscle fibers and of the leucocytes with bismuth and a comparatively small interstitial deposit. After the injection of the oily suspension of the same preparation, muscle fibers and leucocytes at the site of the injection were stained slightly yellow after twenty-four hours, indicating that some of the insoluble material had been dissolved and passed into the cells. After five days, the bismuth particles were surrounded by leucocytes and a few plasma cells.

L. Grumach (48) working with mice and the injections of bismogenol (bismuth salicylate suspended in oil) and spirobismol (bismuth tartrate and bismuth iodoquinine suspended in camphorated oil) never noticed bismuth within the cells, but it is not evident from the publication whether or not attempts were made in this study to stain the bismuth in the histological sections. Up to three weeks, bismuth particles were distinctly visible, but after four weeks, only single bismuth granules could be detected.

After the injection of néotrépol (colloidal metallic bismuth in watery solution), Levaditi and his co-workers (79) found that in the first three days, most of the injected bismuth in form of black granules was imbedded in leucocytes; the partly degenerated muscle fibers also contained some bismuth; and granules were also found in the interstitial tissue. This picture remained almost unchanged for the following three weeks. In case of injections of colloidal metallic bismuth, Gruhzit (47) found that even after seven weeks bismuth was present at the site of the injection.

Levaditi (82) emphasized the fact that these histological staining procedures only visualize the ionized bismuth at the site of the injection, and since tissues which showed no bismuth histologically gave positive results on quantitative analysis, he concludes that some bismuth must have existed in non-ionized form, i.e., presumably bound to proteins.

From these data it follows that all bismuth preparation, injected into the musculature, produce necrobiotic and necrotic processes. The injury is least marked with water and tissue soluble preparations, it is more distinct with water soluble and tissue insoluble preparations, and it is very marked with aqueous and oily suspensions of insoluble bismuth salts. Suspensions of colloidal metallic bismuth seem to produce somewhat less injury than the suspensions of the insoluble salts, but the regenerative processes seem to extend over a very long period. As to the absorption of bismuth from the site of the injection, it appears that the tissue and water soluble preparations are most rapidly absorbed; then follow the water soluble and tissue insoluble preparations, the aqueous and oily suspensions of insoluble salts and finally the suspensions of colloidal metallic bismuth, which show the slowest absorption from the site of the injection.

II. STUDIES ON THE ABSORPTION OF BISMUTH FROM THE SITE OF INJECTION AFTER INTRAMUSCULAR ADMINISTRATION BY THE X-RAY METHOD. The x-ray observation is the method of the choice for studies of the absorption of bismuth in man; it permits the observation of the same deposits at different intervals without any trouble for the patient.

Although the results obtained with the x-ray method may be fairly correct compared with the results obtained afterwards with the analytical methods, as was shown by L. Grumach (48) in rabbits and guinea pigs and by von Oettingen, Todd and Sollmann (115) in rabbits and in cats,

TABLE 1

X-ray studies on the absorption of bismuth from the site of injection

PREPARATION	NUMBER OF OBSERVATION	ABSORPTION	TIME	AUTHOR
			days	
Bismuth salicylate	34	No	14–124	Cole
Bismuth salicylate	5	Complete	28–52	Cole
Bismuth salicylate	5	Complete	45–108	Cole
Bismuth salicylate	1	Incomplete	347	Erdman
Bismuth salicylate		Slight	63	Beinhauer
Bismogenol	2	Little or no	40–44	Cole
Pot. Bismuth tartrate in oil	1	Unabsorbed	210	Erdman
Pot. Bismuth tartrate in oil	27	Complete	6–27	Cole
Pot. Bismuth tartrate in oil	7	Not complete	14–39	Cole
Pot. Bismuth tartrate in oil		Complete	56	Beinhauer
Trépol	1	Large deposit	34	Wolfer
Nadisan		Complete	28–42	H. Müller
Bismuth oleate	—	No visible	12–68	Cole
Coll. met Bi in oil	3	No	44–150	Cole
Néotrépol		Complete	35	Beinhauer
Bicreol		Complete	35	Beinhauer
Na Bi tartrate in water	1	Complete	21	Erdman
Muthanol = $Bi(OH)_3$ in oil		Slow		Lévy-Bing
Bi Iodo Quinine in oil		Complete	28	Beinhauer
Bismudol = Bi phenyl formate		Complete	6	Beinhauer

it is far from being quantitative, but gives rather a rough approximation, and is liable to misinterpretation. Differences in technic will produce differences in intensity of the x-ray shadow, and the same shadow may show a different depth and extension with different positions of the site of the injection, thus simulating a change of the deposit.

Gruhzit (46) asserts that the x-ray shadow may be considerably enlarged by calcification. Small depositions may be entirely overlooked and water and tissue soluble preparations which spread over a large area may form such a fine bismuth layer that it casts no or very little shadow on the x-ray film. Finally, the value of x-ray pictures may be impaired by the necrotic processes produced by some preparations.

X-ray observations on the absorption of bismuth from the site of the injection in animals were performed by Kolle (57), H. Mueller (107), Gordonoff (43), L. Grumach (48), Gruhzit et al. (46), von Oettingen, Todd and Sollmann (115) and others. But since the chemical method gives more reliable results than the x-ray method, only such findings in animal experiments will be discussed further below.

Table 1 illustrates the absorption of bismuth from the site of the injection in man, and is based upon the publications of Cole, Miskdjian and Farmer (27), of Erdmann (37), Beinhauer and Jacobs (12), Wolfer (133), H. Mueller (108), and Levy-Bing (86). From this table it appears that bismuth salicylate is but slowly absorbed from the site of the injection. In thirty-four out of forty-four cases, Cole (27) found no absorption apparent after fourteen to hundred twenty-four days. Erdmann (37) in one case noted a shadow even after three hundred forty-seven days, and Beinhauer and Jacobs (12) reported a slight absorption after sixty-three days. Five of Cole's cases showed completed absorption after twenty-eight to fifty-two days, and five others after forty-five to hundred eight days. With bismuth tartrate in oil the conditions are similar. With colloidal metallic bismuth in oil, furnished by Burroughs, Wellcome Co., Cole found no absorption after forty-four to hundred fifty days, while Beinhauer using néotrépol (colloidal metallic bismuth in aqueous suspension) reported complete absorption after thirty-five days. As may be seen further from this tabulation, the results obtained by the different authors are far from being uniform. The difference may be due in some cases to different sites of the injection; for Cole (27), Gruhzit (46) and others have found that the absorption from the site of the dorsal musculature and from adipose tissue is slower than from the buttocks, due probably to the greater muscular activity in this region, as emphasized again recently by L. Montague (105).

III. Studies on the absorption of bismuth from the site of the injection after intramuscular administration by chemical methods. In order to avoid the fallacies of the x-ray method, several investigators have studied the absorption of bismuth from the site of the

TABLE 2

Absorption of bismuth from the site of injection after intramuscular administration, chemical determination of the unabsorbed bismuth

PREPARATION	APPROXIMATE DOSE OF Bi	ANIMAL	PER CENT OF BISMUTH LEFT AT THE SITE OF THE INJECTION																									ORDER	AUTHOR
			1-2 hours	5 hours	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	13 days	14 days	17 days	18 days	20 days	21 days	25 days	28 days	30 days	32 days	34 days	50 days	100 days	189 days		
Sod. Bismuth Citrate in water	47 mgm. kgm.	Rabbit							,80)					70					40							0				v. Oettingen, Todd, Sollmann
	47 mgm. kgm.	Cat							20																					v. Oettingen, Todd, Sollmann
Sod. Bismuth Tartrate in water	35 mgm. kgm.	Cat												20																v. Oettingen, Todd, Sollmann
Sod. Bismuth Thioglycolate in water	22 mgm.	Dog	0.3	0.2			0.6																							Gruhzit and Sultzaberger
Pot. Bismuth Tartrate in oil	35 mgm. kgm.	Rabbit												98					90							80	70			v. Oettingen, Todd, Sollmann
	33 mgm.	Dog										64								8.9										Gruhzit, Tendick, Sultzaberger
												42.7				7				9.2										
Sod. Pot. Bismuth Tartrate in oil Trépol		Rabbit			70					79					76				83		82									Levaditi et al.
Spirobismol		Guinea pig	52				30					25				16				6		0								L. Grumach

Bismuth Oleate in oil	35 mgm.	Dog													54.3			40.8								Gruhzit, Tendick, Sultzaberger
Bismugenol		Guinea pig	71			59			53	50	42	39						28		10						L. Grumach
Bismuth Salicylate in oil and 10 per cent Creosot and Camphor	76 mgm.	Dog									56.7															Gruhzit, Tendick, Sultzaberger
											13.6				6													
											43.8																	
											16.1				12.2	7.6	1	0.4			0.1	16.5		0				
Same Creosot 2 Camphor 5 Aceton 2 per cent	34.5 mgm.	Dog													5.9			9.6										
Same Camphor 5 Chloretone 5 Ether 2 per cent	37.4 mgm.	Dog											62.5															
													56.4															
Same but Palm kernel oil instead of olive oil		Dog														34.4												
																38.6												
Same without Ether and 1 per cent Chloretone		Dog														14.6												
																5.6												
Bismuth Salicylate in water		Dog									58.9				4.5	6.9		0.03										
Bismuth Hydroxide in water	36 mgm. kgm.	Rabbit											95				88			70						v. Oettingen, Todd, Sollmann		
	36 mgm. kgm.	Cat							100				100															
Bismuth Oxychloride in water		Guinea pig								49	50.1															Lomholt		
Bismuth Oxychloride in oil		Guinea pig							70		26.8					8.62												
																7.08												

injection in animals, by determining chemically the amount of bismuth left at the site of the injection.

Table 2 gives a survey of these determinations after different intervals, as published by L. Grumach (47), Levaditi, Nicolau and Schoen (82), von Oettingen, Todd and Sollmann (115), Gruhzit, Tendick and Sultzaberger (46) (47) and Lomholt (92). In drawing conclusions from this table, three points should be considered: The animal, the bismuth compound, and the method of administration. von Oettingen, Todd, and Sollmann (115) have shown that there is a vast difference between different classes of animals. Using water soluble bismuth citrate, they found that the absorption was much slower in rabbits than in cats; for instance, five days after the intramuscular injection of sodium bismuth citrate (47 mgm. per kgm.) 20 per cent had been absorbed in rabbits, and 80 per cent in cats. Similar differences between different animals are also reported by A. J. Kunkel (63) in relation to the toxicity of the same bismuth preparation, dogs and cats being more susceptible than rabbits, while rats have the highest resistance. The reasons for these racial differences in the rate of absorption, and in the toxicity is not fully elucidated, but their existence emphasizes the fact that precaution is necessary in transferring results obtained in one species to another class of animals or to man. It may be questioned whether the rabbit, which is used most in experimental work in syphilis, is suitable for studying the absorption, excretion and distribution of bismuth.

Table 2 begins with the water soluble tissue insoluble compounds represented by sodium bismuth citrate, sodium bismuth tartrate; and the third is sodium bismuth thioglycolate, which is both water and tissue soluble. The bismuth sodium citrate is much more slowly absorbed in rabbits than in cats. The absorption is not very different from that of sodium bismuth tartrate, but very much slower than that of sodium bismuth thioglycolate, which is practically completed within a few hours. The reason for the slow absorption of the water soluble bismuth citrate and tartrate is probably the formation of insoluble double salts with calcium, as shown by von Oettingen, Sollmann and Schweidt (118), whereas sodium bismuth thioglycolate is said to be soluble in the tissue fluids. Next in the tabulation follow the oil-suspensions of the water soluble preparations, namely, sodium potassium bismuth tartrate and dipotassium bismuth tartrate; then, the oil suspensions of insoluble bismuth preparations, bismuth salicylate, bismuth iodo quinine, bismuth oleate, bismuth hydroxide and bismuth oxychloride. The oily suspensions of the soluble bismuth tartrates show very slow absorption in

rabbits, distinctly faster in guinea pigs and dogs, the latter showing the quickest absorption. Bismuth oleate is decidedly more slowly absorbed in dogs than the soluble bismuth tartrate in oil, for 40.8 per cent were recovered at the end of a twenty-one days period, whereas with tartrate only 9 per cent could be recovered after the same time. The absorption of bismuth salicylate also seems slower in guinea pigs than in dogs. Addition of ether to the injected material seems to delay the absorption, presumably on account of the local irritation of the tissue. The rate of the absorption of the salicylates in dogs is about the same as that of the soluble bismuth tartrate suspension in oil. Salicylate suspended in water seems to be absorbed slightly faster. On the whole, the absorption of the insoluble salts administered in oily suspension is rather irregular, and definite statements could only be made on the basis of larger series of determinations.

The fact that aqueous solutions are more quickly absorbed than watery or oily suspensions seems to indicate that the solubility as well as the viscosity of the preparation is of importance, and that both factors influence the spreading of the injected material. As indicated above, the site of the injection is also important, but probably more so with oily preparations and suspensions than with the water soluble preparations which spread along the intermuscular fascia, even when injected intramuscularly, as was recently pointed out by Shaffer (128). This is demonstrated by the findings of von Oettingen, Todd and Sollmann (115), who found that with intramuscular administration of all aqueous suspension of bismuth hydroxide, thirty per cent of the injected material was unabsorbed after thirty days; whereas after subcutaneous administration even after seventy days, ninety per cent of the injected bismuth could be recovered from the site of the injection. This different behavior of the same compound may perhaps be explained in part by the formation of soaps in the subcutaneous fat.

From these experiments, it may be concluded that water and tissue soluble preparations show the quickest absorption from the site of the injection, next range the water soluble but tissue insoluble preparations; the oily suspensions of the water soluble preparations are absorbed in about the same ratio as the oily suspensions of insoluble preparations; the latter showing a somewhat slower absorption. In transferring the results obtained in animals to man, precaution is advisable, since there are remarkable variations between the different classes of animals as to the rate of absorption of the bismuth deposits from the site of the injection, and as to the toxicity of the same preparation. Finally it has been shown also that the site of the

TABLE 3

Clinical studies on the excretion of bismuth after single intramuscular injections

PREPARATION	DOSE, Bi	TIME OF OBSERVATION	URINE MAXIMAL EXCRETION		TIME	FECES MAXIMAL EXCRETION		TIME	URINE TOTAL EXCRETION		FECES TOTAL EXCRETION		TOTAL EXCRETION		ANALYTICAL METHOD	AUTHOR
	mgm.	*days*	*mgm.*	*per cent*	*days*	*mgm.*	*per cent*	*days*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*		
Sod. Bismuth Tartrate in water	35.5	6	0.55	1.52	3	–	–	–	1.963	5.21	–	–	–	–	Colloid. Bi_2S_3	Sei
Sod. Pot. Bismuth Tartrate in water	50	20	6.20	12.4	2	–	–	–	26.93	53.86	–	–	–	–	Electroscopic	Lomholt
	50	20	5.25	10.5	2	–	–	–	29.66	59.32	–	–	–	–		
	30	14	3.64	12.1	2	–	–	–	14.23	47.43	–	–	–	–		
Sod. Pot. Bismuth Tartrate in oil	70	14	3.68	5.26	2	–	–	–	15.4	22.0	–	–	–	–	Electroscopic	Lomholt
	70	14	3.18	4.54	3	–	–	–	19.61	27.79	–	–	–	–		
Di Pot. Bismuth Tartrate in oil	56	5	0.1	0.18	5	–	–	–	0.27	0.48	–	–	–	–	K.Bi Iodide	Mehrtens, Hanzlik, Marshall and Brown
	100	6	0.8	0.8	6	–	–	–	1.5	1.5	–	–	–	–		
Sod. Pot. Bismuth Tartrate Bismuth Iodo Quinine in camph. oil Spirobismol	3	8	0.6	20	1	0.25	8.3	1	1.81	60.3	0.92	30.7	2.73	91.	K. Bi Iodide	Levy and Selter
	6	7	1.044	17.4	3–4	0.619	10.3	6	5.229	87.1	1.86	31	7.089	118.1		
	12	9	1.786	14.9	8	0.643	5.4	9	7.958	66.3	1.701	14.2	9.659	80.5		
Bismuth Iodo Quinine in oil	90	18	1.07	1.2	14	–	–	–	11.57	12.85	–	–	–	–	Electroscopic	Lomholt
	90	18	1.24	1.4	8	–	–	–	17.99	19.99	–	–	–	–		
Bismuth Iodo Quinine in water	90	21	4.94	5.5	4	–	–	–	42.57	47.30	–	–	–	–		
	90	21	6.32	7.0	3	–	–	–	43.77	48.63	–	–	–	–		
Bismuth Hydroxide in oil	50	14	0.48	0.96	11	–	–	–	3.79	7.58	–	–	–	–	Electroscopic	Lomholt
	50	14	0.44	0.88	2	–	–	–	2.93	5.86	–	–	–	–		
Bismuth Hydroxide in water	65	14	3.29	5.06	7	–	–	–	38.85	47.46	–	–	–	–		
	65	24	2.26	3.47	4	–	–	–	32.64	50.21	–	–	–	–		

Bismuth Hydroxide in 25 per cent glycerol	65	19	3.23	4.97	5	—	—	—	31.77	48.47	—	—	—	—		
	55	14	3.00	5.45	3	—	—	—	24.54	44.61	—	—	—	—		
Bismuth Salicylate in oil	260	8	1.8	0.69	6	—	—	—	6.2	3.48	—	—	—	—	K. Bi Iodide	Mehrtens, Hanzlik et al.
Bismuth Subgallate in oil	200	14	0.92	0.46	13	0.70	0.35	1/2	1.78	0.89	1.74	0.87	3.52	1.76	Colloid. Bi_2S_3	G. Delrue
	200	20	1.40	0.7	11	5.92	2.96	8/9/10	4.42	2.2	9.14	4.57	13.56	7.78		
	200	32	2.85	1.43	8/9	0.54	0.27	3	8.84	4.42	4.93	2.46	13.91	6.85		
	200	14	9.6	4.8	9/10	0.65	0.33	10	17.50	8.75	4.97	2.48	22.47	11.23		
Coll. met. Bismuth	300	4	2	0.66	4	—	—	—	5.2	1.7	—	—	—	—	K. Bi Iodide	Mehrtens, Hanzlik et al.
Bismuthyl	160	55	1.07	0.66	14	0.10	0.06	3	23.34	14.52	1.0	0.62	24.34	15.2	Colloid. Bi_2S_3	G. Delrue
	160	55	2.80	1.75	39/40	0.5	0.31	29	22.44	14.00	1.7	1.2	24.14	15.2		
	180	24	2.10	1.11	17	—	—	—	7.49	4.16	—	—	7.49	4.16		
	240	18	5.22	2.17	2/3	—	—	—	15.10	6.29	—	—	15.10	6.45		
	289	20	8.22	2.8	11	—	—	—	37.88	13.5	—	—	37.88	13.5		
	112	166	1.96	1.75	16	1.3	1.57	10	30.17	26.93	1.78	1.58	31.95	28.5		

injection may determine differences in the rate of the absorption of the bismuth deposits on account of differences in the muscular activity and also because of different properties of the tissue.

STUDIES ON THE EXCRETION OF BISMUTH

In discussing the excretion of bismuth in urine and feces, we have to refer to clinical studies and to animal experiments.

I. CLINICAL STUDIES ON THE EXCRETION OF BISMUTH. The clinical studies were mostly made after single intramuscular injection, some work has been done with repeated intramuscular injections, whereas the study on the excretion of bismuth after intravenous injection is limited to one report. The excretion after those three methods of administration will be discussed separately.

A. Clinical studies on the excretion of bismuth after single intramuscular injections. Table 3, based upon the publications of Mehrtens, Hanzlik, Marshall and Brown (102); G. Delrue (32), Sei (125), S. Lomholt (90) (91) and Levy and Selter (87), gives a survey of the clinical work on the excretion of bismuth after *single* intramuscular injections in man. It states the preparation, its dose, the duration of the observation, the level of the maximal urinary and fecal excretion (i.e., the highest daily excretion in milligram and in per cent of the injected bismuth), the time in which this occurred, the total urinary and fecal excretion, and the total excretion in milligram and in per cent of the injected bismuth.

a. Water soluble preparations. The first section refers to *water soluble preparations*, i.e., to experiments performed with different types of soluble bismuth tartrate, which are extensively used in the treatment of syphilis. The first preparation employed by Sazerac and Levaditi (121–124) was a watery solution of alkaline bismuth tartrate; however, it was too irritant; oily suspensions of different bismuth tartrates were substituted, but recently a watery solution of bismuth tartrate was put on the market, which seems to be less painful. The various bismuth tartrates show great differences of bismuth content, ranging, according to Warren (131), from 30.75 to 74.44 per cent; even preparations of the same source may show considerable variations. von Oettingen, Ishikawa and Sollmann (116), (117) have shown that slight modifications in the method of preparing these compounds, may lead to different preparations. For this reason there is no assurance as to whether bismuth tartrates of the same nomenclature used by different observers are identical.

Sei (125) reports on the excretion of bismuth after the intramuscular

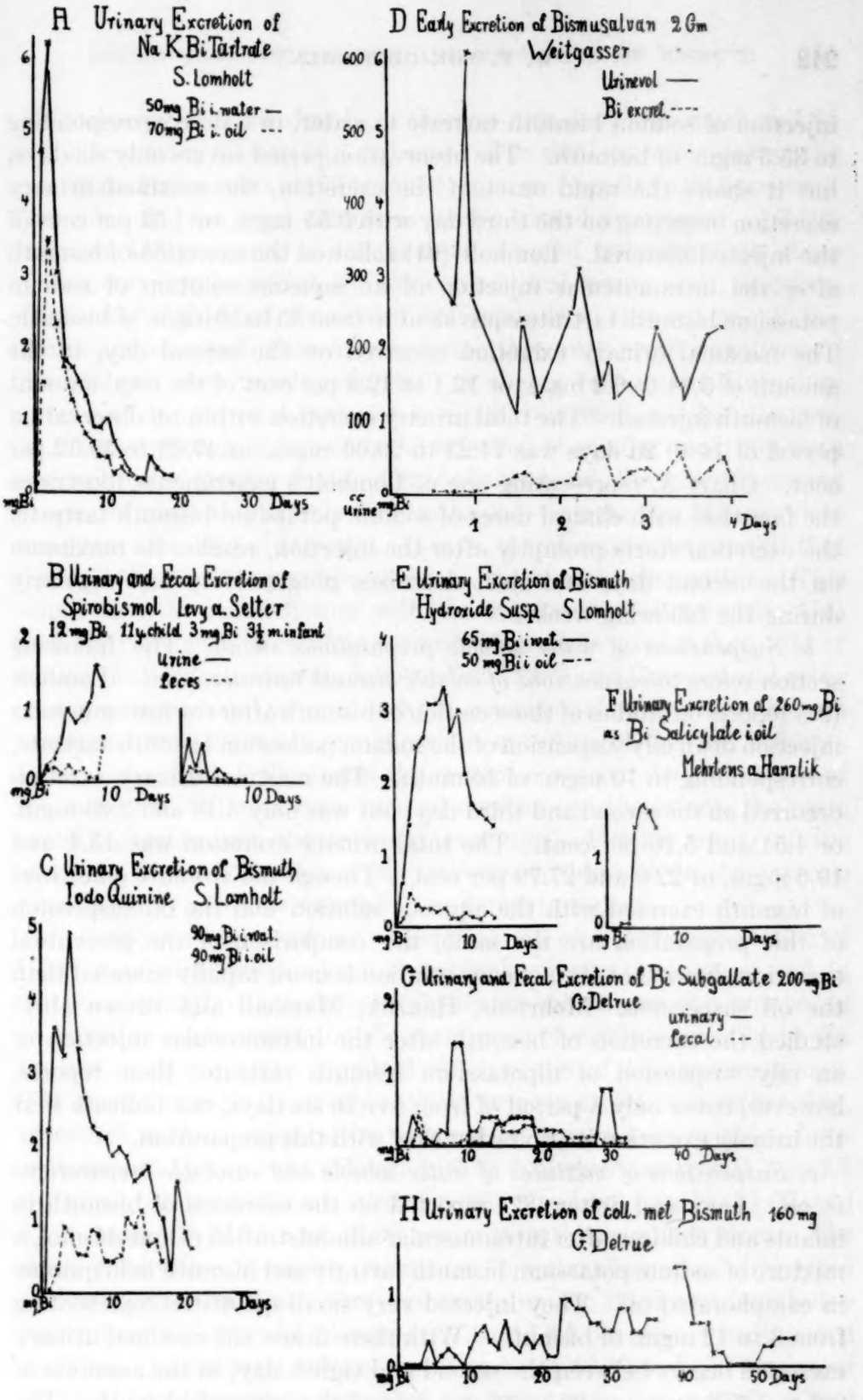

Chart 1. The excretion of bismuth after therapeutic doses of various preparations with single intramuscular injections in man.

injection of sodium bismuth tartrate in water, in a dose corresponding to 35.5 mgm. of bismuth. The observation period covers only six days, but it shows the rapid onset of the excretion, the maximal urinary excretion occurring on the third day with 0.55 mgm. or 1.52 per cent of the injected material. Lomholt (91) followed the excretion of bismuth after the intramuscular injection of an aqueous solution of sodium potassium bismuth tartrate equivalent to from 30 to 50 mgm. of bismuth. The maximal urinary excretion occurred on the second day, in the amount of 3.64 to 6.2 mgm. or 12.1 to 12.4 per cent of the total amount of bismuth injected. The total urinary excretion within an observation period of 14 to 20 days was 14.23 to 29.66 mgm. or 47.43 to 59.32 per cent. Chart A, representing one of Lomholt's experiments, illustrates the fact that with clinical doses of sodium potassium bismuth tartrate, the excretion starts promptly after the injection, reaches its maximum on the second day, and then decreases progressively and regularly during the following weeks.

b. Suspensions of water soluble preparations in oil. The following section refers to *suspensions of soluble bismuth tartrates in oil.* Lomholt (91) reports on studies of the secretion of bismuth after the intramuscular injection of an oily suspension of the sodium potassium bismuth tartrate, corresponding to 70 mgm. of bismuth. The maximal urinary exretion occurred on the second and third day, but was only 3.18 and 3.68 mgm. or 4.54 and 5.26 per cent. The total urinary excretion was 15.4 and 19.6 mgm. or 22.0 and 27.79 per cent. Though the absolute quantities of bismuth excreted with the aqueous solution and the oil suspension of this preparation are the same, the comparison of the percentual excretion shows that the aqueous solution is more rapidly excreted than the oil suspension. Mehrtens, Hanzlik, Marshall and Brown (101) studied the excretion of bismuth after the intramuscular injection of an oily suspension of dipotassium bismuth tartrate; their reports, however, cover only a period of from five to six days, but indicate that the urinary excretion is also rather slow with this preparation.

c. Suspensions of mixtures of water soluble and insoluble preparations in oil. Levy and Selter (87) reported on the excretion of bismuth in infants and children after intramuscular administration of spirobismol, a mixture of sodium potassium bismuth tartrate and bismuth iodo quinine in camphorated oil. They injected very small quantities representing from 3 to 12 mgm. of bismuth. With these doses, the maximal urinary excretion occurs between the second and eighth day, in the amounts of 0.6 to 1.786 mgm. or 20 to 15 per cent of the injected bismuth. The

total urinary excretion after from three to nine days is as much as 1.81 to 7.958 mgm. or 60.3 to 66.3 per cent. The total excretion during the same period ranges from 2.73 to 9.659 mgm. or from 91 to 80.5 per cent. The value for the total excretion, in the second experiment, of 7.089 mgm. or 118 per cent of the amount injected indicates that these experiments are impaired by some analytical error, and, therefore, conclusions should only be drawn cautiously from this report. If the results are accepted, they may indicate that very small quantities of bismuth are more rapidly excreted, or that a certain small absolute amount of the injected material is rapidly excreted, so that the percentual excretion is higher with the injection of smaller quantities than with the use of greater doses. On the other hand, the infant receiving 3 mgm. of bismuth was only 3½ months old, whereas the 12 mgm. dose was given to a child of 11 years. It may, therefore, also be possible that the more juvenile organism shows a more rapid elimination. This would seem in contradiction with the statement of Bentivoglio (14) that the resistance of children towards bismuth is greater than that of adults, but Drouet, Marfan, Lereboullet, Debré and Crémieu and other clinicians recommend precaution in the treatment of infants with bismuth preparations. Chart 1B compares the excretion of the two children. In the older child, it illustrates the prompt onset and the gradual rise of the excretion, followed by a gradual decrease; in the infant, the more rapid beginning and the irregular excretion. Further study of the excretion of small doses and the excretion of bismuth in the juvenile organism may give interesting and important results.

d. Suspensions of insoluble preparations in oil. The next section of table 3 refers to suspensions of insoluble preparations in oil and in water. Bismuth iodo quinine is the active constituent of a number of preparations, advocated for the treatment of syphilis, usually in the form of an oil emulsion. Lomholt (91) has studied its excretion in man. The results on table 3 indicate that the urinary excretion is rather slow, inasmuch as the maximal urinary excretion occurred after eight to fourteen days in the amount of from 1.07 to 1.24 mgm. or from 1.2 to 1.4 per cent; the total urinary excretion was also low, namely, 11.57 and 17.99 mgm. or 12.85 and 19.99 per cent within eighteen days. The same preparation, however, suspended in water showed a fairly rapid excretion, the maximal urinary excretion on the third and fourth day amounting to 6.32 and 4.94 mgm. or 7.0 and 5.5 per cent of the injected material, and the total urinary excretion being 42.57 and 43.77 mgm. or 47.3 and 48.6 per cent at the end of the twenty-one day period. Chart

1C represents two of Lomholt's experiments and illustrates the difference in the rate of excretion between the oil and the watery suspension. It shows that the injection of aqueous suspensions is followed by a rapid increasing, and then gradually decreasing excretion, whereas the oily suspension shows a slow onset and with moderate variations lasts over a long period. A comparison of charts 1A and 1C shows that the bismuth tartrate and the bismuth iodo quinine in water are excreted in a very similar way; the oily suspensions, however, show a marked difference. In the case of the tartrate, the excretion of the oily suspension is of the same type as that of the aqueous solution, whereas with bismuth iodo quinine, the aqueous and the oily suspension show a different type of excretion.

I. Weitgasser (132), using bismosalvan, a suspension of bismuth iodo quinine in oil, has made a special study of the onset of the bismuth excretion. He found that the first trace of bismuth appeared in the urine at the end of the first day. A comparison of the bismuth excretion and the urine excretion does not reveal any relation, at least not during the first two days, as may be seen from chart 1D.

Bismuth hydroxide in suspension in oil or in colloidal solution in water, is the constituent of several antiluetics. Lomholt (90) (91) published comparative studies on the excretion of suspensions in oil, and in water with and without 25 per cent of glycerol. With oily suspensions, the maximal urinary excretion occurred on the second and eleventh day, and amounts to 0.48 and 0.44 mgm. or 0.88 and 0.96 per cent; the total urinary excretion after fourteen days being 3.79 and 2.93 mgm. or 7.6 and 5.9 per cent of the injected material. With aqueous suspensions, the maximal urinary excretion was higher, but was also relatively late; on the fourth and seventh day, the total urinary excretion was 38.9 and 32.6 mgm. or 47.5 and 50.2 per cent after fourteen and twenty-four days respectively. The suspension containing 25 per cent of glycerol had practically the same excretion as the aqueous suspension. Chart 1E illustrates the difference in the excretion of the aqueous and the oily suspension. The aqueous suspension resembles somewhat the tartrate type, though the decrease in the excretion is slower, but the oily suspension shows a very uniform and low bismuth level in the urine.

On the excretion of bismuth after single intramuscular injections of oily suspensions of bismuth salicylate in men, only the reports of Mehrtens, Hanzlik, Marshall and Brown (102) are available. These cover a period of only eight days. The low maximal urinary excretion

on the seventh day, and the low total excretion of 6.2 mgm. or 3.48 per cent of the injected material at the end of the eight day period, indicate the slow excretion which is illustrated in chart 1F.

G. Delrue (32) reports on excretion studies after the intramuscular administration of *bismuth subgallate suspended in oil.* With this preparation, the maximal urinary excretion was rather low and varied between 0.92 and 9.6 mgm. or 0.5 and 4.8 per cent after from eight to thirteen days. Corresponding to this, the total urinary excretion was also low, namely, from 1.78 to 17.5 mgm. or 0.89 to 8.75 per cent after from fourteen to thirty-two days. Delrue determined also the bismuth excreted in the feces, which reached its maximal value in one to ten days with from 0.54 to 5.92 mgm. or 0.3 to 2.9 per cent; the total being 1.74 to 9.14 mgm. or 0.87 to 4.57 per cent. The great irregularity in the urinary and in the fecal excretion is especially noteworthy in these experiments, and raises the question whether there may have been some analytical error, as for instance, the presence of iron.

Chart 1G illustrates one of Delrue's experiments with the great irregularity of the excretion; it seems especially surprising that at times the fecal excretion is greater than the urinary. Delrue is inclined to attribute this to the constitution of the bismuth subgallate, but this appears questionable.

e. Suspensions of metallic bismuth. The last section of table 3 refers to experiments with metallic colloidal bismuth. From Delrue's (32) experiments it is evident that the metallic bismuth is only very slowly excreted. The excretion started on the second or third day, and even hundred-sixty-six days after the injection of 112 mgm. of bismuth, only 31.95 mgm. or 28.5 per cent of the injected material were excreted. The total excretion was likewise slow. After the injection of 240 mgm. of bismuth, 15.1 mgm. or 6.45 per cent were excreted after eighteen days, and after the injection of 289 mgm., 37.88 mgm. or 13.15 per cent in twenty days. Chart 1H representing one of Delrue's experiments, illustrates the wavelike excretion, the peaks always being followed by a more or less marked depression. Comparison of chart 1H and 1A reveals the striking difference in the excretion after the injection of the colloidal metal, and of the water soluble salt in aqueous solution; the colloidal bismuth curve showing a prolonged excretion extending over seven weeks, while the tartrate is characterized by a sharp rise, and rather rapid decrease of the excretion, which is practically finished at the end of three weeks.

In most of the publications only the urinary excretion was determined,

because the determination of the bismuth in feces requires more time on account of the longer digestion of large amounts of organic material, and partly also because the determinations in feces are more subject to analytical errors, as for instance, those produced by the presence of iron. Lomholt assumes that the fecal excretion goes parallel with the urinary excretion, and amounts to one-half of the latter or to one-third of the total excretion. This may hold true for some preparations, but it cannot be considered as a general rule. According to Delrue, the ratio between urinary and fecal excretion after the administration of colloidal metallic bismuth varies between 23 to 1 and 4 to 1; with dermatol it is even more irregular, namely, 1 to 2 and 4 to 1. Levy and Selter (87) found variations between 2 to 1 and 5 to 1, and Mehrtens, Hanzlik and Marshall (102) found only very small quantities or traces of bismuth in feces. Our personal experience indicates that the ratio of urinary and fecal excretion is very inconstant, and we do not feel justified in assuming any definite relation between the two.

From these studies it becomes evident that several factors are involved in the excretion of bismuth after intramuscular injection. The most important are the solubility, and the difference between aqueous and oily suspensions. The water soluble preparations are promptly excreted, and the excretion after having reached the maximum, decreases rather rapidly. When suspended in oil, the soluble preparations are excreted more slowly. Insoluble preparations suspended in water are excreted more rapidly than those suspended in oil, and it seems that the type of the excretion of the oil suspension depends also upon the chemical constitution of the bismuth compound. The slowest excretion occurs with the colloidal metallic bismuth preparations. These findings are in accordance with the results obtained with the different preparations as to their absorption from the site of the injection. It has also been suggested that age may play a rôle, and Hanzlik, Bloomfield, Stokton and Wood (49) *point out that the condition of the circulatory and excretory apparatus of the organism is of importance, since they found the excretion of bismuth perceptibly smaller in edematous patients than in normal persons.*

B. Clinical studies on the excretion of bismuth after repeated intramuscular injections. Since bismuth is generally administered in courses of several injections, studies on the excretion of bismuth after serial injections are important to determine whether or not, and to which extent an accumulation of bismuth is produced in the organism. As yet, very few reports on such studies in man are available, namely, those of Sei (125) and of Mehrtens, Hanzlik, Marshall and Brown (102). The determinations in

Sei's reports are incomplete, since on some days no determinations were made. The studies of Mehrtens and Hanzlik cover only a very short period, but as may be seen from chart 2A and 2B, they indicate a rapid accumulation of the bismuth in the organism.

C. Clinical studies on the excretion of bismuth after intravenous injections. Only comparatively few preparations of bismuth are suited for intravenous injection, and even these are liable to produce more or less serious accidents, and have no advantage over the intramuscular administration in the treatment of syphilis. Clinical studies on the excretion of bismuth after intravenous injections are therefore rare,

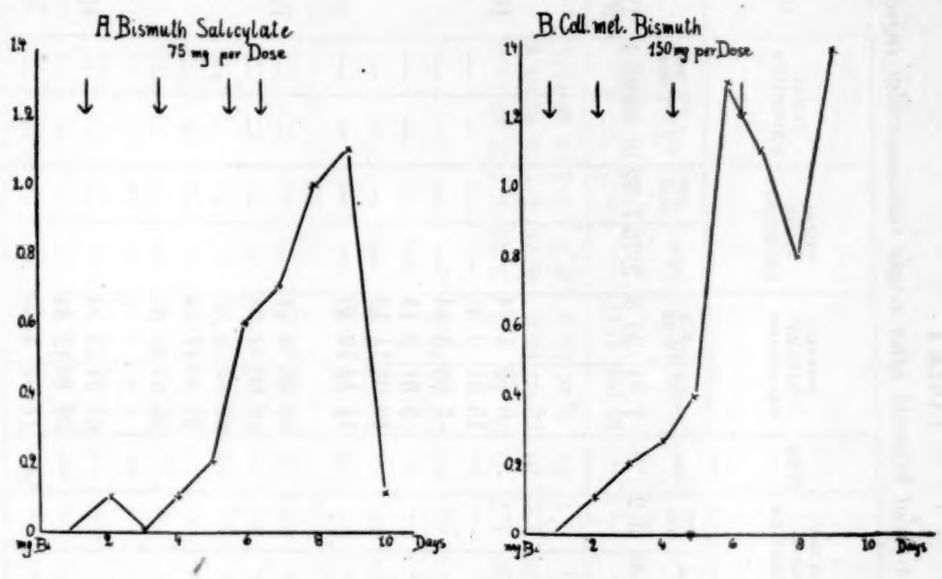

Chart 2. The excretion of bismuth after therapeutic doses of various preparations with repeated intramuscular injections in man.

and only the report of Memmesheimer (100) gives some data. After the repeated injections of 40 mgm. of salluen, a preparation of unknown composition, with the total amount of 640 mgm. of bismuth, he found a maximal urinary excretion of 5.0 mgm. or 0.78 per cent on the fifth and the thirty-second day, and a total urinary excretion of 84.3 mgm. or 13.2 per cent, and of 115 mgm. or 17.9 per cent on the fiftieth day. The ratio between urinary and fecal excretion was about 8 to 1.

ANIMAL STUDIES. *A. Animal studies after single intramuscular injections.* Animal experiments are generally used for the study of the excretion of bismuth and especially in those cases in which the distribution of bismuth in the organism and the deposits at the site of the injec-

TABLE 4

Studies on the excretion of bismuth after single intramuscular injections

PREPARATION	DOSE	TIME OF OBSERVATION	MAXIMAL URINARY EXCRETION		TIME	MAXIMAL FECAL EXCRETION		TIME	TOTAL URINARY EXCRETION		TOTAL FECAL EXCRETION		TOTAL EXCRETION		ANIMAL	ANALYTICAL METHOD	AUTHOR
	mgm. Bi	*days*	*mgm.*	*per cent*	*days*	*mgm.*	*per cent*	*days*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*			
Sod. Bismuth thio glycolate in water	37.5	17	3.74	9.99	1	1.20	3.2	2	7.81	20.8	2.75	7.32	10.56	28.1	Dog	K Bi Iodide	Gruhzit a. Sultzaberger
Sod. Bismuth thio sulfate in water	550	3	4.46	0.81	1	—	—	—	6.86	1.24	—	—	—	—	Rabbit	K Bi Iodide	Leonard
	670	4	5.14	0.76	2	—	—	—	15.91	2.38	—	—	—	—			
	200	6	3.76	1.88	1	—	—	—	7.58	3.84	—	—	—	—			
	180	5	2.67	1.48	2	—	—	—	5.67	3.15	—	—	—	—			
	90	11	1.90	2.11	4	—	—	—	10.01	11.12	—	—	—	—			
	90	14	2.16	2.36	2	—	—	—	11.14	12.40	—	—	—	—			
Sod. Pot. Bismuth tartrate in water	394	3½	12.20	3.10	1	—	—	—	16.55	4.21	—	—	—	—	Rabbit	K Bi Iodide	Leonard
	312	4	9.89	3.17	1	—	—	—	14.90	4.78	—	—	—	—			
	238	1½	17.55	7.37	½	—	—	—	20.94	8.80	—	—	—	—			
	120	10	6.33	5.27	8	—	—	—	21.33	17.63	—	—	—	—			
	103	14	6.37	6.19	1	—	—	—	26.81	26.03	—	—	—	—			
Sod. Bismuth citrate in water	320	22	5.17	1.61	2	—	—	—	61.31	22.34	—	—	—	—	Rabbit	K Bi Iodide	Leonard
	270	14	7.86	2.49	14	—	—	—	39.10	12.34	—	—	—	—			
	262	8	2.79	1.06	5	—	—	—	11.07	4.50	—	—	—	—			

Di Pot. Bismuth tartrate in oil	233	6	3.06	1.31	2	—	—	—	8.16	3.50	—	—	—	—	Rabbit	K Bi Iodide	Leonard
	217	7	1.52	0.70	4	—	—	—	6.50	2.99	—	—	—	—			
	200	6	1.63	0.83	1	—	—	—	7.94	4.03	—	—	—	—			
	190	6	4.36	2.32	3	—	—	—	7.88	4.19	—	—	—	—			
	168	15	2.10	1.25	2	—	—	—	17.63	10.48	—	—	—	—			
	140	15	3.20	2.31	5	—	—	—	16.22	11.70	—	—	—	—			
	135	15	11.60	8.21	7	—	—	—	43.69	33.55	—	—	—	—			
	97	15	2.79	2.78	5	—	—	—	25.50	26.30	—	—	—	—			
	77		6.14	7.98	6	—	—	—	32.50	42.24	—	—	—	—			
Bismuth oleate in oil	380	11	2.06	0.54	3	—	—	—	6.95	1.83	—	—	—	—	Rabbit	K Bi Iodide	Leonard
	280	14	2.29	0.82	2	—	—	—	15.85	5.66	—	—	—	—			
	220	14	2.40	1.09	3	—	—	—	14.17	6.44	—	—	—	—			
	210	14	4.50	2.15	14	—	—	—	22.15	10.6	—	—	—	—			
Bismuth salicytate in oil	216	54	3.10	1.4	6	3.60	1.7	6	20.19	9.3	5.49	2.5	25.68	11.8	Dog	K Bi Iodide	Gruhzit, Tendick and Sultzaberger
	72	24	0.95	1.7	6	0.76	1.0	19	12.07	16.8	4.75	6.6	16.82	23.4			
	72	52	1.30	1.8	4	0.5	0.7	17	9.65	13.4	2.5	3.4	12.15	16.8			
Coll. met. Bismuth	1,120	12	3.50	0.30	6	—	—	—	13.06	1.17	—	—	—	—	Rabbit	K Bi Iodide	Leonard
Neotrepol	190	22	3.15	1.75	3	—	—	—	36.24	19.20	—	—	—	—			

tion are determined at the end of the experiment; being generally assumed that the animal experiment permits conclusions on the conditions in the human organism. It has, however, been demonstrated that the rate of the absorption from the site of the injection varies in different classes of animals, and it seems probable that similar differences may exist in regard to the excretion. Besides the following will show that the doses usually employed in animal experiments are very much higher than those used clinically, so that they may produce side actions, which ordinarily do not occur in man.

Table 4 gives a survey of experiments performed on animals with single intramuscular injections, based on the reports of C. Leonard (72–74), Gruhzit and Sultzaberger (47), Gruhzit, Tendick and Sultzaberger (45). The arrangement is the same as that of table 3.

a. Tissue and water soluble preparations. Gruhzit and Sultzaberger (47) reported on the excretion of bismuth in dogs after the intramuscular injection of 37.5 mgm. of bismuth in form of bismuth thioglycolate, a water soluble preparation. The maximal urinary excretion occurred at the end of the first day in the amount of 3.74 mgm. or 9.99 per cent, the total urinary excretion amounting to 7.8 mgm. or 20.8 per cent after seventeen days; the preparation is excreted rather rapidly, in accordance with the velocity of the absorption from the site of the injection. Chart 3 A illustrates one of these experiments. It shows that after a rapid initial excretion within the first two days, the excretion decreased perceptibly, and only small quantities or traces were excreted during the following weeks. Gruhzit and Sultzaberger also determined the fecal excretion as 2.75 mgm. or 7.32 per cent. Since the total urinary excretion is 20.8 per cent, the ratio of urinary to fecal excretion is about 3 to 1.

b. Water soluble preparations. Sodium bismuth thiosulphate is easily soluble in water, but also easily decomposed with the formation of bismuth sulphide. According to Kolle (57), the latter has about the same toxicity as the insoluble bismuth tartrate; and while sodium thiosulphate has a very low toxicity, bismuth thiosulphate is highly nephrotoxic. Leonard (74) studied the excretion of bismuth after the intramuscular injection of various doses, as given in table 4. In these experiments, doses from 180 to 550 mgm. must be considered as highly toxic, and even the smaller dose of 90 mgm. equivalent to 50 mgm. per kgm., which did not cause the death of the animal within fourteen days, was found to produce considerable tubular necrosis. This toxicity was also reflected in the urinary excretion of bismuth, because the maximal urinary excre-

tion occurred with all animals within the first and second day, but with the higher dose it was considerably lower than with the smaller one, namely, 4.46 mgm. or 0.81 per cent as compared with 2.16 mgm. or 2.36 per cent. The total urinary excretion was also distinctly smaller with the larger doses, although these results are somewhat obscured by the differences in the time of the observation. Chart 3 B illustrates one of Leonard's experiments in which 50 mgm. of bismuth were injected: After a short, marked initial rise, the excretion dropped down and remained at a rather low level. With larger doses, the excretion was usually very marked in the beginning, but was generally reduced to mere traces or even to zero toward the end of the experiment. From this, it appears that the pathological changes of the kidney interfere with the excretion of the metal, which is corroborated by the fact that the animal died with uremic symptoms.

Leonard (70) also reported on the excretion of bismuth after intramuscular injections of sodium potassium bismuth tartrate in aqueous solution. He found that the dose of 200 mgm. per kgm. was highly toxic, since the animals died with uremic symptoms within 1½ to 5 days. With half the dose, one animal died after ten days, another was killed after fourteen days. While the first group showed extensive tubular necrosis, the latter showed only occasional areas of light necrosis. From this, it appears that the smaller dose is also within the toxic range. This toxicity is likewise reflected in the urinary excretion of bismuth which was distinctly smaller with the larger dose even in view of the fact that the observation period was considerably reduced. Chart 3 C gives the results obtained with the small dose in which the animal was killed at the end of fourteen days. It shows a very high initial excretion, followed by a series of remissions. Comparison with chart 1 A illustrates the distinct contrast of the excretion in man with a clinical dose, and the excretion in the rabbit with a nephrotoxic dose. The repeated remissions found in these experiments are considered by Leonard as a series of crises of the kidney, the nature of which is unknown, but incomplete micturition may also give similar variations, and Leonard did not state whether the daily urine was voided spontaneously or whether it was collected by catheterization.

Sodium bismuth citrate, in doses of 125 mgm., corresponding to 85 mgm. of bismuth per kgm. is (according to Leonard, 70) of low or no toxicity as compared with the water soluble sodium potassium bismuth tartrate in corresponding doses. However, larger doses of 300 mgm. corresponding to 200 mgm. of bismuth, are more toxic, showing evidence

of beginning pathological changes of the kidney. Since Leonard did not give the excretion of bismuth in larger doses in extenso, it is not represented in table 4. The excretion of the smaller dose follows fairly closely the amount of bismuth injected. Chart 3 D which illustrates one of the experiments with the smaller dose, shows the rapid excretion over a long period, but with remissions similar to other water soluble preparations.

c. Suspensions of water soluble preparations in oil. The third section of table 4 refers to water soluble preparations in oil suspension, confined to the experiments of Leonard (73) with an oily suspension of dipotassium bismuth tartrate. According to Leonard, the maximal tolerated dose of dipotassium bismuth tartrate is 150 mgm. corresponding to 75 mgm. of bismuth per kgm., and the minimal nephrotoxic dose is 100 mgm. corresponding to 50 mgm. per kgm. Table 4 shows that four of the experiments were performed with lethal doses, one within the nephrotoxic range, and two below this limit. The most characteristic findings with lethal and sublethal doses again concern the kidney showing necrotic reactions. Accordingly, the total urinary excretion in the first group was rather low, namely, 6.5 to 7.88 mgm. or 2.99 to 4.19 per cent within six days, and the maximal urinary excretion is also lower than with the smaller dose. The values found with nephrotoxic doses are distinctly higher and the two smallest doses show a total urinary excretion of 25.5 and 32.5 mgm. or 26.3 and 42.24 per cent. Chart 3 E illustrating one of Leonard's experiments, shows a type of curve similar to that obtained in clinical studies (chart 1 A), but at first the excretion occurs with very high remissions, which may indicate some stimulation of the kidney function. Toward the end of the experiment, the excretion seems to be distinctly impaired, which also seems to confirm this assumption.

d. Suspensions of water insoluble preparations in oil and in water. The fourth section refers to oily suspensions of water insoluble bismuth preparations. The first preparation of this group is Oleobi, an oily suspension of bismuth oleate, used by Leonard (73), who found that a dose containing 200 mgm. of bismuth is close to the maximal tolerated dose, and even half this amount is decidedly nephrotoxic. All of his experiments were therefore within the nephrotoxic range and this is also indicated by the low urinary excretion of 6.95 to 22.15 mgm. or 1.83 to 10.6 per cent within eleven to fourteen days. Although it was shown that the absorption of bismuth oleate from the site of the injection is rather low, namely, about 59 per cent, after twenty-one days, chart 3 F shows that with the nephrotoxic dose the same irregularity of excretion is observed as with dipotassium bismuth tartrate.

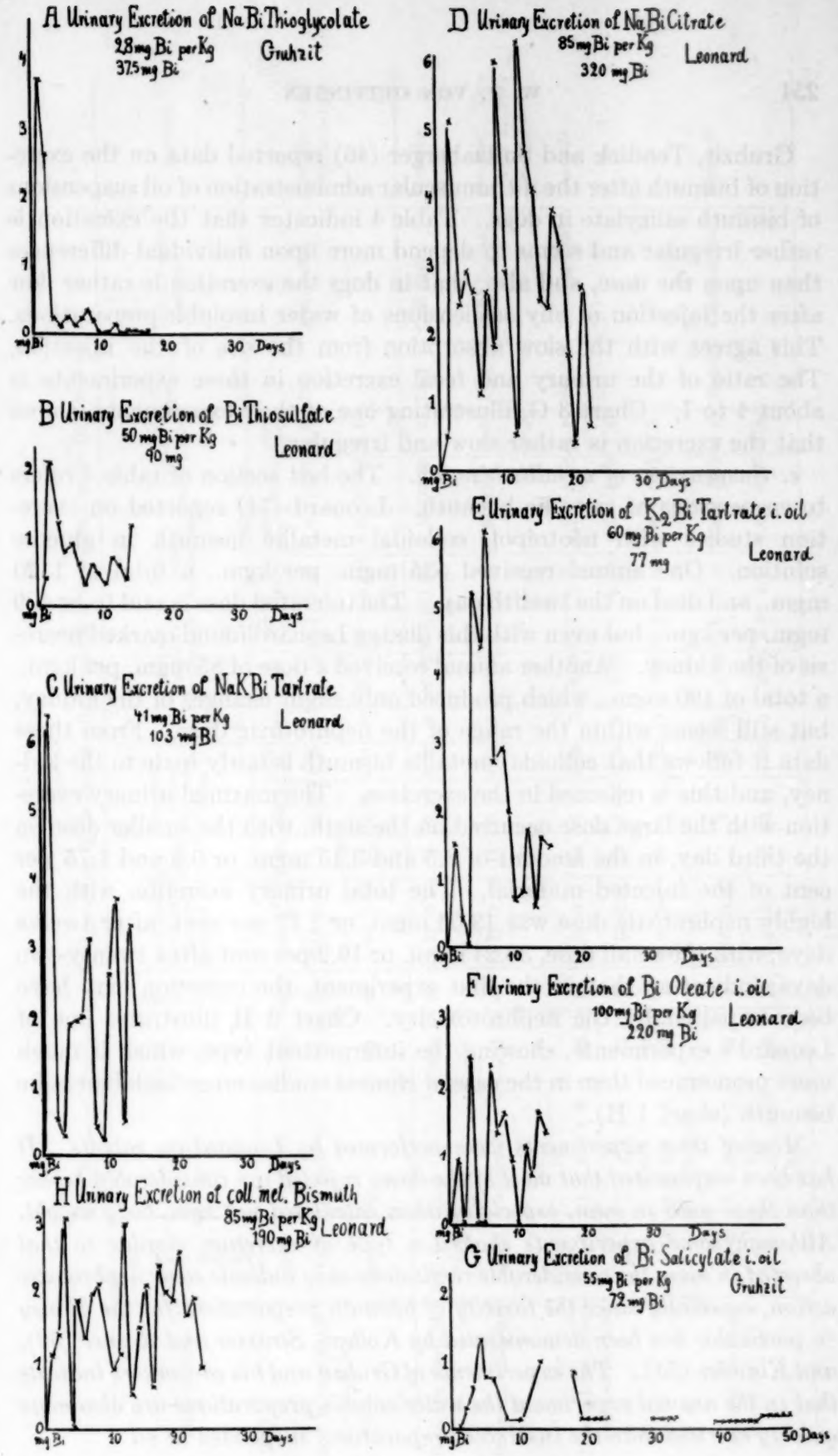

Chart 3. The excretion of bismuth after single intramuscular injections of various preparation in animals.

Gruhzit, Tendick and Sultzaberger (46) reported data on the excretion of bismuth after the intramuscular administration of oil suspensions of bismuth salicylate in dogs. Table 4 indicates that the excretion is rather irregular and seems to depend more upon individual differences than upon the dose, and also, that in dogs the excretion is rather low after the injection of oily suspensions of water insoluble preparations. This agrees with the slow absorption from the site of the injection. The ratio of the urinary and fecal excretion in these experiments is about 4 to 1. Chart 3 G, illustrating one of their experiments, shows that the excretion is rather slow and irregular.

e. Suspensions of metallic bismuth. The last section of table 4 refers to suspensions of metallic bismuth. Leonard (74) reported on excretion studies with néotrépol, colloidal metallic bismuth in glucose solution. One animal received 535 mgm. per kgm., a total of 1120 mgm., and died on the twelfth day. The tolerated dose is said to be 400 mgm. per kgm., but even with this dosage Leonard found marked necrosis of the kidney. Another animal received a dose of 85 mgm. per kgm., a total of 190 mgm., which produced only slight changes of the kidney, but still seems within the range of the nephrotoxic dose. From these data it follows that colloidal metallic bismuth is fairly toxic to the kidney, and this is reflected in the excretion. The maximal urinary excretion with the large dose occurred on the sixth, with the smaller dose on the third day, in the amount of 3.5 and 3.15 mgm. or 0.3 and 1.75 per cent of the injected material. The total urinary excretion with the highly nephrotoxic dose was 13.06 mgm. or 1.17 per cent, after twelve days; with the small dose, 36.24 mgm. or 19.2 per cent after twenty-two days; indicating that in the first experiment, the excretion may have been impaired by the nephrotoxicity. Chart 3 H illustrates one of Leonard's experiments, showing the intermittent type, which is much more pronounced than in the case of clinical studies on colloidal metallic bismuth (chart 1 H).

Most of these experiments were performed by Leonard on rabbits. It has been emphasized that most of the doses injected are considerably higher than those used in man, especially when calculated per kgm. body weight. Although some experiments showed a type of excretion similar to that observed in man, the considerable remissions may indicate some nephrotoxic action, especially since the toxicity of bismuth preparations for the kidney in particular has been demonstrated by Kolbert, Strasser and Rosner (56), and Klauder (55). The experiments of Gruhzit and his co-workers indicate that in the animal experiment the water soluble preparations are also more quickly excreted than the insoluble preparations suspended in oil.

TABLE 5

Animal studies on the excretion of bismuth after repeated intramuscular injections

PREPARATION	SINGLE DOSE, Bi	INTERVAL	TOTAL DOSE, Bi	TIME OF OBSERVATION	MAXIMAL URINARY EXCRETION		TIME	MAXIMAL FECAL EXCRETION		TIME	TOTAL URINARY EXCRETION		TOTAL FECAL EXCRETION		TOTAL EXCRETION		ANIMAL	ANALYTICAL METHOD	AUTHOR
	mgm.	*days*	*mgm.*	*days*	*mgm.*	*per cent*	*hours*	*mgm.*	*per cent*	*days*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*			
Sod. Bismuth thio glycolate in water	6.25	10 in 25	62.5	49	2.13	3.4	6	0.86	1.38	7	9.73	15.5	8.68	13.9	18.41	29.6	Dog	KBi Iodide	Gruhzit and Sultzaberger
	10.5	10 in 25	105	49	1.64	1.56	*days* 17	1.39	1.31	9	18.58	17.7	9.38	8.9	27.96	26.6			
Bismuth oleate in oil	0.1; 0.2; 0.3; 0.4	1. 2. 3. 4.	1000	28	8.12	0.8	8	7.04	0.70	8	102.75	10.27	71.94	7.19	174.69	17.47	Dog	Coll Bi_2S_3	Kuerthy
Bismuth salicylate in oil	19.55	within 57	234.6	32	2.77	1.18	14	0.50	0.21	14	14.06	6	1.34	0.57	15.4	6.5	Dog	K Bi Iodide	Gruhzit, Tendick a. Sultzaberger
Bismuth iodo quinine viochine in oil	2.65	1. 2. 3. 4. 5. 6. 7.	18.55	11	0.39	2.1	10	0.31	1.66	7	2.17	11.7	1.15	6.1	3.62	19.4	Rabbit	Electroscopic	Lomholt
	2.5	1. 2. 3. 4. 5. 6. 7. 8. 9.	22.5	10	0.63	2.8	10	0.08	0.36	6	1.29	5.7	0.47	2.1	1.76	7.8			
	1.55	1. 2. 3. 4. 5. 6. 7. 8. 9. 10.	15.5	14	0.22	1.4	10	0.08	0.52	10	1.47	9.5	0.59	3.8	2.06	13.3			
Bismuth hydroxide in oil	3.80	1. 2. 3. 4. 5. 6. 7. 8. 9.	34.2	15	0.49	1.43	7	0.42	1.2	15	4.37	12.8	2.35	6.8	6.72	19.6	Rabbit	Electroscopic	Lomholt
	16.0	1. 2. 3. 4. 5. 6. 7. 8.	128	12	0.87	0.7	10	0.34	0.26	9	5.06	3.9	1.31	1.0	6.37	4.9			

B. Animal studies after repeated intramuscular injections. There are only a few publications on the subject of the excretion of bismuth after repeated intramuscular injections. They are illustrated by table 5, based on the publications of Kuerthy (60), Lomholt (89), Gruhzit and Sultzaberger (47), and Gruhzit, Tendick and Sultzaberger (46). One of five preparations used is water soluble, and four are oily suspensions of insoluble salts.

a. Studies on the excretion in animals after repeated intramuscular injections of water soluble preparations. Gruhzit and Sultzaberger (47) reported on the excretion of bismuth after repeated intramuscular injections of sodium bismuth thioglycolate in dogs, the total dose being 62.5 and 105 mgm. During forty-nine days, they found a total excretion of bismuth of 18.41 and 27.96 mgm. or 29.6 and 26.6 per cent, the slightly smaller excretion following the larger dose. The ratio between urinary and fecal excretion was about 2 to 1.

b. Studies on the excretion of bismuth in animals after repeated intramuscular injections of insoluble preparations suspended in oil. Kuerthy (60) working with bismuth oleate, oleobi Roche, found after repeated intramuscular injections of a total of 1000 mgm. in dogs, a total excretion of 174.69 mgm. or 17.47 per cent resulted after twenty-one days; the ratio between urinary and fecal excretion being about 13 to 1.

Gruhzit, Tendick and Sultzaberger (46) report on excretion studies after repeated injections of bismuth salicylate suspended in oil, given in twelve doses of 19.55 mgm. each over a period of fifty-one days. The total excretion of the injected bismuth, at the end of fifty-four days was 15.4 mgm. or 6.5 per cent; the ratio between urinary and fecal excretion was about 10 to 1.

Lomholt (89) studied the excretion in rabbits after the repeated injection of bismuth iodo quinine and bismuth hydroxide, presumably suspended in oil. With the first preparation, the total excretion was from 1.76 to 3.62 mgm. or 7.8 to 19.4 per cent within ten to fourteen days; the ratio between urinary and fecal excretion being about 3 to 1. With the bismuth hydroxide suspension, the excretion at the end of fifteen and twelve days was 6.72 and 6.37 mgm. or 19.6 and 4.9 per cent, the larger dose showing the smaller percentual excretion. The ratio between urinary and fecal excretion was 2 to 1 with the smaller and 4 to 1 with the larger dose.

These experiments do not permit a comparison with those made in the previous series with the same preparation, because either the animals used are of different classes or because the period of the observation differs too

widely. However, they indicate that considerable amounts of bismuth may accumulate in the organism after repeated injections.

C. Animal studies on the excretion of bismuth after oral administration. Mehrtens, Hanzlik, Marshall and Brown (102) did not detect bismuth in the urine of man after the oral administration of 3 to 15 grams of bismuth subnitrate. Mueller and Kuerthy (112) working with dogs found that after the administration of a single dose of 40 grams of bismuth subcarbonate, 11.07 mgm. or 0.03 per cent were excreted in the urine within ten days. After repeated fractional doses totaling to 70 grams of bismuth oxide, the urinary excretion was 36.88 mgm. or 0.05 per cent after twelve days, and after several doses of a total of 8 grams of bismuth oxide, the total urinary excretion was 16.27 mgm. or 0.2 per cent after eleven days, the larger dose producing the lower excretion. After administration of 50 grams of bismuth subcarbonate to patients with gastric superacidity, Dorner and Weingartner (35) recovered as much as 173.8 mgm. of bismuth in the urine. If confirmed, this would indicate, that the absorption after oral administration of bismuth depends largely on the acidity of the stomach.

D. Animal studies on the excretion of bismuth after percutaneous administration. Kuerthy (60) reported one experiment on the excretion of bismuth after bismocutan, a preparation of unknown composition containing free bismuth ions, was rubbed into the skin of a dog. He found a total urinary excretion of 1.69 per cent after twenty-two days. The ratio of urinary to fecal excretion was about 1 to 16. This considerable fecal excretion (27.8 per cent) arouses the suspicion whether the animal may have swallowed some material by licking with the tongue, especially since no precautions to exclude this source of error are mentioned. There is no evidence that the whole amount was completely rubbed into the skin. These data therefore seem to need further confirmation.

STUDIES ON THE DISTRIBUTION OF BISMUTH IN THE ORGANISM.

I. DISTRIBUTION OF BISMUTH IN THE ORGANISM OF MAN. In comparing the amount of bismuth absorbed from the site of the injection and the quantity of bismuth excreted with urine and feces, it becomes evident that the absorbed bismuth is not completely excreted, the excretion being slower than the absorption or mobilization of the deposits. A part of the bismuth must therefore be stored in the organs.

There are only very few reports on the distribution of bismuth in

man from autopsy material after the injection of bismuth preparations.

Table 6, based upon the publications of Mueller, Blass and Kratzeisen (110), of Autenrieth (5), Levy and Selter (87), and Munk (113), gives a résumé of these studies. These data, meager as they are, show that the kidney contains the largest amount of the bismuth, and that the liver and the spleen come next; this agrees with the more extensive data from animal experiments. Besides these, we have the report of Lemay and Jaloustre (70), who detected bismuth in the brain of two patients who had undergone a bismuth treatment.

TABLE 6

Distribution of bismuth in different organs in man (mgm. Bi per gram tissue)

	PREPARATION			
	Spirobismol	Bismugenol	Na K Bi Tartrate in oil	Bi Hydroxide in oil
Author	Levy and Selter	Autenrieth	Müller et al.	Munk
Age	Infant	Adult	Infant	Adult
Dose, mgm. Bi	36		13	5,130
Time of Observation	1 day	35 days		11 months
Organ				
Heart	0.0042	—	—	—
Spleen	0.0046	0.00063	—	0.018
Kidney	0.0211	0.0053	0.05	0.065
Liver	0.0235	0.00004	0.008	0.0105
Muscle	—	—	—	—
Lungs	0.00305	—	—	—
Brain	—	—	0.015	—

II. Distribution of bismuth in the organism of animals. Most experiments on the distribution of bismuth in animals have been performed on rabbits and dogs; with a few experiments on guinea pigs. Most of the studies concern intramuscular, a few intravenous and subcutaneous injections and oral administration.

A. Distribution of bismuth after intramuscular injections. A survey on the distribution of bismuth in rabbits after the intramuscular administration of various bismuth preparations is given in table 7, based on the publications of Levaditi, Sanchis—Bayarri, Schoen and Mannin (84), Levaditi, Nicolau, Girard and Mannin (82), Leonard (75), Fabre and Picon (38), (39), Lomholt (89), and Levy and Selter (87).

a. Distribution of bismuth after intramuscular injections of water soluble preparations. The first section deals with water soluble preparations. Leonard (75) using an aqueous solution of sodium bismuth citrate found that twenty-four hours after the administration of a dose corresponding to 82 mgm. per kgm., the average tissue percentage was in the following sequence: Adrenals, spleen, kidney, liver, followed by heart, brain, bone, muscle and lungs. After the injection of the same dose of sodium potassium bismuth tartrate, the average order was: Kidney, spleen, adrenals, liver, brain, lung and heart. In comparing these two series, it becomes evident that with the citrate, the adrenals contain much more bismuth than with the tartrate, and in the latter case, kidney and liver contain larger quantities than with the citrate.

b. Distribution of bismuth after intramuscular injections of water-soluble preparations suspended in oil. The next section refers to water soluble preparations suspended in oil. Levaditi and his co-workers (82) reported on the distribution of bismuth four, six or seven days after intramuscular injections of sodium bismuth tartrate suspended in oil in a dose corresponding to 60 mgm. of bismuth per kgm. The sequence of the organs according to their bismuth content per gram tissue is: Kidney, lung, heart, brain, liver and muscle. The surprisingly large bismuth content of the lung and of the heart suggests that some of the material may accidentally have entered a ruptured vein. Leonard (75) reported on similar experiments with dipotassium bismuth tartrate in oil, in the dose of 72 mgm. of bismuth per kgm. One of the three animals died after six days, the two others were killed at the end of the second week. The sequence in the first was: Kidney, spleen, lung, liver, and heart. In the two others: Kidney, spleen, liver and lung. A fourth animal which had received a total dose of 222.9 mgm. of bismuth, was killed after forty-eight hours. The uterus was found to contain 0.01 mgm. of bismuth per kgm., the kidney, 0.034 mgm., and the pancreas none. Comparison of these results obtained after the intramuscular injection of bismuth tartrate suspended in oil, with those found after the intramuscular injection of the bismuth tartrate in aqueous solution, reveal no striking differences between these two preparations.

c. Distribution of bismuth after intramuscular injection of insoluble preparation suspended in oil. The third section refers to the intramuscular administration of insoluble bismuth preparations suspended in oil. Levy and Selter (87) reported on the intramuscular injection of spirobismol, an oily suspension of sodium bismuth tartrate and bismuth iodo quinine in oil, in different doses, after six, thirteen, and seventeen days.

The spleen showed the greatest bismuth content followed by the kidney, heart and liver. The surprisingly high value of bismuth in the lung of experiments 2 and 3, and the high figures of the bismuth concentration in the spleen of the same experiments, suggest some technical

Distribution of bismuth in different organs after intramuscular ... in rabb...

	Sodium Bismuth Citrate in water			Sod. Pot. Bismuth Tartrate in water			Sod. Pot. Bismuth Tartrate in oil*			Di Pot. Bismuth Tartrate in oil			...	...
Author	Leonard			Leonard			Levaditi et al.			Leonard				...and Selte...
Dose per kgm	82	82	82	82	82	82	60	60	60	75	75	75	...	200
Total dose	206	206	215	238	222	222	147	185	128	217	168	138	...	540
Time of observation	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours	4 days	6 days	7 days	7 days	14 days	14 days	...	6 days
Organ														
Brain	0.00985	0.00336	0.00341	0.0095	0.0024	0.0061	—	0.004	—	—	—	—	...	0.555
Spinal Cord	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Adrenals	0.160	0.100	0.0223	0.0265	0.0294	0.0462	—	—	—	0.000	0.00	0.00	...	—
Heart	—	0.00476	0.00358	0.0058	0.0067	0.005	—	—	—	0.0013	0.00	0.00	...	0.0242
Spleen	0.0874	0.00521	0.0359	0.0952	0.0011	0.0834	0.048	0.005	—	0.0245	0.0353	0.0689	...	0.194
Blood	0.00727	0.00175	0.00217	0.0011	0.0059	—	0.002	—	—	—	—	—	...	—
Saliv. Gland	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Stomach	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Small Intestine	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Cecum	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Large Intestine	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Kidney	0.0283	0.0212	0.0438	0.0963 0.0702	0.0635 0.0680	0.0829 0.0932	0.044	0.024	0.028	0.0666	0.682	0.0790	...	0.0354
Bladder	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Liver	0.00528	0.00680	0.00384	0.0182	0.0109	0.0141	0.004	0.002	0.001	0.0019	0.0363	0.0727	...	0.0192
Muscle	0.00666	0.0125	0.00333	—	—	—	—	—	—	—	—	—	...	—
Inj. Muscle	—	—	—	0.817	0.272	—	17.5	8	9	—	—	—	...	—
Testicle	—	—	—	—	—	—	0.003	0.002	—	—	—	—	...	—
Skin	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Bone	0.00311	0.0392	0.0390	—	—	—	—	—	—	—	—	—	...	—
Teeth	—	—	—	—	—	—	—	—	—	—	—	—	...	—
Lung	0.00376	0.0101	0.00294	0.0116	0.0063	0.0078	0.030	0.016	—	0.0023	0.000	Trace	...	0.040

accident during the injection. Lomholt (89) reported on the intramuscular injection of bismuth iodo quinine and of bismuth hydroxide suspended in oil. His results are somewhat lower than those of Leonard with water soluble preparations; this might be explained by the longer observation period. In these experiments, the order of the organs according to their bismuth content was: Kidney, heart, lung and liver.

d. Distribution of bismuth after the intramuscular injection of lipoid soluble preparations dissolved in oil. The last section of table 7 refers to lipoid soluble preparations dissolved in oil. Fabre and Picon (38) reported on the intramuscular injection of bismuth campho carbonate

*in rabbits (mgm. Bi per 1 gram moist organ, * refers to dry organ)*

PREPARATION			Bismuth Iodo Quinine in oil		Bismuth Hydroxide in oil		Bismuth Camph. Carbon in oil		Bismuth Camph. Carbonate in oil*					bas. Carboxy-β-ethyl-nonoate of Bismuth in oil*			
			Lomholt		Lomholt		Fabre and Picon		Levaditi et al.					Levaditi et al.			
	200	200	—	—	—	—	—	—	10	25	5	5	20	1	2.2	6.6	25
	540	552	22.5	18.55	128	34.2	500	600	23	46.25	10	11.5	52	2.3	5	16.5	75
	6 days	6 days	10 days	11 days	12 days	15 days	10 days	26 days	14 days	15 days	19 days	27 days	57 days	85 days	85 days	52 days	107 days
	0.555	0.0453	—	—	—	—	0.009	0.0075	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	0.0545	—	—	—	—	—	—	—	—	—	—
	0.02427	0.01572	0.0025	0.0012	0.0014	0.0133	0.078	0.0065	—	—	—	—	—	—	—	—	—
	0.194	0.09846	—	—	—	—	0.0615	—	—	0.001	0.007	0.006	—	0.06	—	—	—
	—	0.00371	0.0003	0.0004	0.00014	0.003	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	0.0005	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	0.0034	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	0.003	0.070	0.010	0.032	0.002	0.00	0.00	0.002	0.011
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	0.0354	0.07832	0.014	0.02	0.0217	0.0294	0.1517	0.0315	0.080	0.120	0.070	0.070	0.017	0.00	0.00	0.012	0.09
	—	—	—	—	—	—	0.018	0.023	—	—	—	—	—	—	—	—	—
	0.01928	0.03531	0.0010	0.0010	0.0009	—	0.059	0.0453	0.006	0.026	0.008	0.007	0.001	0.00	0.00	0.006	0.002
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	0.0012	0.285	0.5	0.040	0.150	0.130	0.009	0.007	0.008	—
	—	—	—	—	—	—	—	0.1757	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	0.0444	0.0154	—	—	—	—	—	—	—	—	—
	0.0405	0.00604	0.0027	0.0011	0.0027	0.0041	0.0126	0.0164	—	0.002	0 004	0.002	—	0.00	0.00	—	0.002

in dose of 500 and 600 mgm.: The animals had been killed after ten and twenty-six days. In one animal, the bismuth content of the kidney was by far the highest, but in the other experiment, it was surpassed by that of the liver. The studies of Levaditi and his co-workers (84) with the same preparation, indicate that the kidney is very rich in bismuth, while the bismuth content of the liver decreases with the time

of the survival of the animal after the administration of the bismuth. Their results show further that, in the rabbit, the cecum is very rich in bismuth; the same holds true for the results obtained after the intramuscular administration of basic carboxy-beta-ethyl-nonoate of bismuth.

Table 7, as a whole, shows that the largest amount of bismuth is usually found in the kidney after intramuscular injections; this is also confirmed by the findings of Meseke (103); Biro (16) and Lessevici-Dragunesco (78). The distribution of bismuth in the other organs exhibits considerable more variation, which become more manifest when their bismuth content is expressed as percentage of the bismuth content of the kidney.

Table 8 gives a survey of the experiments, discussed so far and calculated on this basis. It reveals considerable variation in the results with the same preparation by the same author, and under very similar conditions. It may be doubted, however, whether the determinations, which require different amounts of material and yield different quantities of bismuth are generally reliable.

Before closing this subject, it should be mentioned that the testicles contain only very small amounts of bismuth, namely, from 0.002 to 0.003 mgm. of bismuth per gram of tissue; and according to Levaditi (83), and Levaditi and Girard (81), the values in syphilitic lesions are also very small, ranging from 0.005 to 0.01 mgm. per gram tissue, so that there is evidently no accumulation of bismuth in the syphilitic lesion.

Levy and Selter (87) discussing the high bismuth content found in the liver of one infant, suggest that the bismuth content of the liver is rather high in the beginning, and that it is reduced rather quickly with the lapse of time, which seems to be confirmed by the experiments of Levaditi with bismuth campho carbonate. Leonard (75), however, is inclined to assume just the opposite behavior. Therefore, this question requires further study.

Table 9 represents similar experiments performed on dogs. The first section gives the reports of Gruhzit and Sultzaberger (47) on the distribution of bismuth after the intramuscular injection of sodium bismuth thioglycolate. The amounts of bismuth found in the different organs are rather small, in accordance with the rapid absorption of this drug from the site of the injection and the rapid excretion of this preparation. The kidney has the highest bismuth content, in the dog as well as in the rabbit. This holds true also for the experiments of Gruhzit, Tendick and Sultzaberger (46) with the injection of bismuth

TABLE 8

Distribution of bismuth in different organs in relation to the bismuth content of the kidney (= 100)

	PREPARATION																													
	Sod. Bismuth Citrate in water			Sod. Pot. Bismuth Tartrate in water			Sod. Pot. Bismuth Tartrate in oil			Di Pot. Bismuth Tartrate in oil			Spirobismol				Bism. Iodo. Quinine in oil		Bismuth Hydroxide in oil		Bism. Camph. Carb. in oil		Bismuth Camph. Carbonate in oil					Bicarboxy-β-ethyl-nonoate	
Author	Leonard			Leonard			Levaditi et al.			Leonard			Levy and Selter				Lomholt		Lomholt		Fabre and Picon		Levaditi et al.					Levaditi	
Dose per kgm	82	82	82	82	82	82	60	60	60	75	75	75	70	100	200	200	—	—	—	—	—	—	10	25	5	5	20	6.6	25
Total dose	206	206	215	238	222	222	147	185	128	217	168	138	255	300	540	552	22.5	18.55	128	34.2	500	600	23	46.25	10	11.5	52	16.5	75
Time of observation	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	4 days	6 days	7 days	7 days	14 days	14 days	13 days	17 days	6 days	6 days	10 days	11 days	12 days	15 days	10 days	26 days	14 days	15 days	19 days	27 days	57 days	52 days	107 days
Organ																													
Brain	35	16	18				—	17	—	—	—	—	24	147	—	58	—	—	—	—	6	22	—	—	—	—	—	—	—
Adrenals	565	471	51	26	43	49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35	—	—	—	—	—	—	—	—
Heart	—	22	8	6	10	5	109	21	—	2	—	—	37	120	70	20	18	6	6	45	50	19	—	—	—	—	—	—	—
Spleen	309	24	82	99	2	90	5	—	—	37	52	87	75	780	550	126	—	—	—	—	40	—	—	1	10	9	—	—	—
Blood	26	8	5	1	9	—	—	—	—	—	—	—	2	9	—	5	2	2	1	10	2	—	—	—	—	—	—	—	—
Saliv. Gland	—	—	—	—	—	—	—	—	—	—	—	—	64	912	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Cecum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38	60	14	46	17	16	122
Kidney	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Bladder	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11	70	—	—	—	—	—	—	—
Liver	19	32	9	19	16	15	10	9	5	3	53	92	4	50	54	45	13	5	4	—	38	133	75	22	11	10	8	50	22
Muscle	24	59	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Testicle				—	—	—	7	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bone	11	185	89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	—	—	—	—	—	—	—	—
Teeth	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	45	—	—	—	—	—	—	—
Lung	13	48	7	12	9	8	69	67	—	3	—	—	2	173	114	8	19	5	12	14	8	48	—	2	5	3	—	—	22

TABLE 9

Distribution of bismuth in different organs after intramuscular injection in dogs (mgm. Bi per gram tissue)

	PREPARATION													
	Sodium Bismuth Thioglycolate in water							Bismuth Salicylate in oil						Bismuth Camph. Carb.
Author	Gruhzit and Sultzaberger							Gruhzit, Tendick and Sultzaberger						Fabre and Picon
Dose per kgm	2	2	2	2	28	0.75	0.5	—	—	—	—	—	—	27 × 50
Total dose	17.2	23.6	24.6	26.3	37.5	105	62.5	76	76	72	76	76	216	1350
Time of observation	6 hours	1 day	1 day	3 days	17 days	25 days	25 days	8 days	14 days	24 days	28 days	42 days	189 days	59 days
Organ														
Brain	0.0004	0.00023	0.0004	0.00013	0.00065	0.00025	0.00030	0.006	0.0067	0,0.008	0.0063	0.0044	0.004	0.002
Heart	0.0003	0.0002	0	0.0	0.0	0.00018	0.0	—	—	—	0.0064	0.0014	0.0071	0.01
Spleen	0.0005	0.0028	0.0153	0.0107	0.00082	0.0035	0.0017	0.0233	0.0067	0.0025	0.0067	0.089	0.0071	—
Blood	0.0062	0.0004	0.0005	0.0046	0.0	0.0	0.0	—	—	—	—	—	—	0.005
Saliv. Gland	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Small Intestine	0.00111	0.0004	0.0005	0.00083	0.0008	0.0004	0.0017	—	—	0.0005	—	—	—	0.03
Kidney	0.0203	0.0152	0.0154	—	0.0051	0.0089	0.0081	0.0380	0.0480	0.0160	0.0096	0.0140	0.0057	0.0413
Liver	0.0004	0.0011	0.0010	0.0014	0.0012	0.0005	0.0008	0.0059	0.0030	0.0024	0.0038	0.0049	0.0023	0.0302
Muscle	0.0023	0.0005	0.0004	—	0.00048	0.00023	0.00032	0.0091	0.0060	0.0005	0.0052	0.0016		0.0044
Inj. Muscle	0.0014	0.00094	0.0013	—	—	0.00052	0.00035	0.1027	0.0456	0.0792	0.017	0.0079	0.0028	1.09
Skin	—	—	—	—	—	0.0002	0.0005	—	—	—	—	—	—	—
Bone	0.0015	0.0017	0.0015	0.0016	—	0.0019	0.0026	—	—	—	—	—	—	—
	0.0011	0.0023	0.0016	0.0013		0.0023	0.0036							
Teeth	—	—	—	—	—	—	—	—	—	—	—	—	—	0.04
Lung	0.0003	0.0005	0.0004	0.0005	0.0009	0.0004	0.0004	—	0.0032	0.0009	0.0027	0.0042	—	0.01

salicylate suspended in oil. The findings of Fabre and Picon (38) with bismuth campho carbonate dissolved in oil, show a similar distribution.

Summarizing the intramuscular injection of the various bismuth preparations in rabbits and dogs, it may be stated that the bismuth is distributed through the whole organism, the largest quantity generally being found in the kidney. The distribution among the other organs varies considerably, but the existing data do not permit any conclusions as to the factors influencing the distribution. The experimental work performed so far with rabbits and dogs does not reveal any significant differences between these animals, as to the distribution of bismuth in the organism.

B. Distribution of bismuth in different organs after intravenous administration. Table 10 is based on the publications of Fabre and Picon (39); Sei and Weise (127); Masson (97); Levaditi, Schoen, Girard and Mannin (82), Akamatsu (1); Memmesheimer (100); and Autenrieth (5).

The first section refers to water soluble preparations as ammonium bismuth citrate, sodium bismuth tartrate, bismuth cacodylate and Wismulen. The distribution of bismuth with intravenous injections appears to be rather irregular, and kidney, liver or spleen may show the highest bismuth content. The next group, the lipoid soluble preparations, is represented by bimuth campho carbonate dissolved in oil. In the two experiments reported by Fabre and Picon, the distribution was rather irregular. The same holds true for the findings of Akamatsu with colloidal metallic bismuth, and of Memmesheimer with salluene, a colloidal bismuth preparation of unknown composition.

With intravenous injections, the difference in the distribution of bismuth in different organs presumably depends in part upon the chemical character of the preparation used, especially on the ability to form precipitates with the serum, and in part upon the stability of these suspensions and of colloidal solutions, because in both cases the particles would be filtered off mechanically from the circulating blood in the large organs of the hematopoietic system: The liver, the spleen and the lungs. In this respect, the experiments of Fabre and Picon are of interest. With intravenous injection into the ear vein, the lungs contain the highest amount of bismuth, while with intravenous administration into the mesentery vein, the liver contains the major amount, and the lungs not more than usually detected in this organ. These studies with intravenous injections illustrate the accidents which may be encountered with this method of administration, namely, embolism and irregular distribution.

C. Distribution of bismuth in the organism after subcutaneous injections.

TABLE 10

Distribution of bismuth in different organs after intravenous injection into rabbits (mgm. Bi per 1 gram tissue)

	PREPARATION																	
	Ammonium Bismuth Citrate			Na Bi Tartrate	Na Bi Tartrate	Sod. Pot. Bismuth Tartrate			Bismuth cacodylate		Wismulen	Bismuth Camph. Carbon.		Coll met. Bismuth		Salluene	
Author	Fabre and Picon			Sei and Weise	Masson	Levaditi et al.			Fabre and Picon		Autenrieth	Fabre and Picon		Akamatsu		Memmesheimer	
Single dose	18 x 5	11 x 12.5				16.5	25.65		35 x 5								
Total dose, mgm. Bi	90	137	407	45	26.7			216	175	407		20	10	19.80	14.85	320	160
Time of observation				4 hours	2 days	2 days	33 days	83 days			6 hours	4 days*	1 hour†	½ hour	8 hours	2½ days	1 day
Organ																	
Brain	—	0.005	0.002	0.0015	—	0.001	0.004	0.038	0.014	0.007	0.013	0.008	—	—	—	0.009	0.003
Spinal Cord	—	—	—	—	—	—	—	—	—	—	0.011	—	—	—	—	—	—
Heart	0.01	—	—	0.0039	—	—	—	—	—	—	—	—	0.022	—	—	—	0.07
Spleen	—	—	—	0.2363	0.01	0.024	0.050	0.054	—	—	—	—	—	—	—	1.0	1.0
Blood	—	0.006	0.005	0.0038	—	—	—	—	0.006	trace	0.005	0.003	—	—	—	—	—
Stomach	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0316	0.0316	—	—
Small Intestine	—	—	—	0.0057	—	—	—	—	—	—	—	—	—	—	0.0154	0.027	0.011
Cecum	—	—	—	—	—	—	—	—	—	—	—	—	—	0.029	0.0667	0.016	0.005
Large Intestine	—	—	—	0.0103	—	—	—	—	—	—	—	—	—	0.0066	0.048	—	—
Kidney	0.0733	0.244	0.254	0.137	0.0066	0.003	0.010	0.161	0.0418	0.08	0.014	0.038	—	0.07	0.200	0.14	0.05
Liver	0.015	0.067	0.02	0.033	0.0166	0.004	0.034	0.053	0.011	0.0406	0.007	—	0.116	0.270	0.180	0.73	0.108
Muscle	0.001	0.004	0.0015	0.0005	—	—	—	—	0.002	0.001	—	—	—	—	—	—	—
Lung	0.004	0.007	0.001	0.0063	—	0.004	0.030	0.080	0.013	0.003	—	0.894	0.02	—	—	0.19	0.08

* In ear vein.

† In mesenteric vein.

M. Akamatsu (1) reported on the distribution of bismuth after subcutaneous administration of sodium bismuth tartrate and sodium bismuth thioglycolate in rabbits.

As may be seen from table 11, the largest amount was found in the kidney, and next follows the liver. In the intestinal tract, the cecum contained the largest quantities, large intestine, stomach and small intestine following in the order given. The bismuth content of the brain after the administration of sodium bismuth thioglycolate is surprisingly high, in comparison with the results reported in dogs after the

TABLE 11

Distribution of bismuth in different organs after subcutaneous administration in rabbits (mgm. Bi per 1 gram tissue)

	PREPARATION				
	Sodium Bismuth Tartrate			Sodium Bismuth Thioglycolate	
Author	Akamatsu			Akamatsu	
Total Dose Bi_2O_3	103	135	90	52.2	54.6
Time of Observation	1 hour, 10 minutes	3 hours 30 minutes	11 hours	1 hour, 50 minutes	3 hours, 20 minutes
Organ					
Brain	—	trace	trace	0.2	0.175
Heart	—	trace	trace	—	0.660
Liver	0.100	0.160	0.150	0.120	0.120
Stomach	0.033	0.0556	0.042	0.084	0.080
Small Intestine	0.013	0.033	0.024	0.055	0.052
Cecum	0.076	0.0857	0.1047	0.100	0.111
Large Intestine	0.0556	0.080	0.061	0.100	0.92
Kidney	0.182	0.520	0.267	0.720	0.836

intramuscular administration by Gruhzit and Sultzaberger (47). The distribution of bismuth in the intestinal tract may be understood in the light of the results of Meyer and Steinfeld (104), that the deposition of bismuth in the intestinal canal depends largely upon the presence of hydrogen sulphide. Since the latter exists in greater quantities in the large intestine, a greater deposit of bismuth may be expected than in the other parts of the intestinal tract. The larger amount of bismuth found in the cecum agrees with the high values found by Levaditi in the same organ. This may be explained by the reports of J. A. Bargen, A. E. Osterberg, and F. C. Mann (8), that bismuth is actually excreted

into the isolated colon after oral administration of sodium bismuth tartrate dissolved in water.

D. Distribution of bismuth in the organism after oral administration. Kuerthy (60) reported on the distribution of bismuth in a dog which twelve weeks before had been fed large doses of bismuth carbonate.

The intestine contained the largest amount of bismuth followed closely by the liver, while kidney and spleen contained considerably less. These data may be explained by the fact that the absorbed material must pass through the liver before entering the circulation, so that the conditions resemble those of Fabre and Picon's experiment with intravenous injection into the mesentery vein.

III. Histological studies on the distribution of bismuth in different organs. Several investigators have attempted to demonstrate histologically the presence of bismuth in various organs, using

TABLE 12

Distribution of bismuth in different organs after oral administration of bismuth oxy carbonate to dogs (mgm. Bi per 1 gram tissue) (Kuertly)

Brain	0.012
Spleen	0.147
Kidney	0.102
Intestine	0.065
Liver	0.108

the methods that were discussed under the histological studies of the site of the injection.

G. Komaya (58) and L. Califano (21) after intravenous injections of colloidal preparations, diasprosal (col. bismuth hydroxide) and colloidal metallic bismuth, found that the Kupffer cells of the liver, and the reticular cells of the spleen and of the lungs were rich in bismuth, while the follicular tissue was entirely free from it. In the kidney, the bismuth was stored within the cells of the tubuli contorti, and none could be detected in the vasa colligentia, the loops and the glomeruli. This distribution of the colloidal bismuth is the same as that of other colloidal particles injected into the circulation, and the distribution is, therefore, probably more characteristic of the colloidal phase than of the bismuth. Similar experiments with intraperitoneal injections of néotrépol, performed by Aloiz Chura (25), showed bismuth only in the large intestine. E. Fränkel (40) injected a total quantity of 216 mgm. of bismuth in form of spiro-bismol during the course of two months. The animal

died finally with considerable loss of weight, and in this animal also the Kupffer cells were filled with black precipitates of bismuth.

THE PERMEABILITY OF THE PLACENTA FOR BISMUTH

In connection with the distribution of bismuth in the organism, the question may be discussed whether or not bismuth passes from the maternal organism into the fetus. Leonard and Love (76) did not find forty-eight hours after sublethal doses of dipotassium bismuth tartrate in oil detectable amounts of bismuth in the fetus; but after the injection

TABLE 13

Appearance of bismuth in the blood of dogs after intramuscular injection of dipotassium bismuth tartrate according to Leonard and Seibert

Weight of dog	Bi injected	Bi per kgm.	Bi in 1 cc. of blood								
			2 hours	4 hours	6 hours	24 hours	48 hours	17 days	28 days	37 days	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
12	500	40	0	0	0	0.001	0.0015	0.0008	0.0003	0	No massage
7	290	40	0.0015	0.002	0.001	0.0009	0	0.0005	—	—	Massage
			24 hours	44 hours	48 hours	50 hours	70 hours	72 hours	6 days	30 days	
8	400	50	0.0025	0.002	0.003	0.008	0.0009	0.0009	—	—	
13	600	50	0.0009	0.002	0.002	0.001	0.0009	0.0007	0.0005	0	
			12 hours	16 hours	18 hours	24 hours	14 days	21 days	29 days		
17	850	50	0.0018	0.002	0.0012	0.0012	0.009	0	0		
22	1,100	50	0.002	0.0019	0.0011	0.0011	—	—	—		

of single lethal doses of sodium bismuth tartrate and dipotassium bismuth tartrate into pregnant animals, the bismuth passed through the placenta. The bismuth which had reached the fetus forty-eight hours after the injection into the maternal organism was detectable only in the kidneys; but the quantities were so small that it required the kidneys of a whole liter to yield detectable amounts. According to Leonard, the fetal kidney contains one-half to one times the amount of the maternal kidney per gram of tissue. Leonard further found that bismuth does not accumulate in the placenta, and that the quantities of bismuth found there are hardly greater than those found in the uteri

of non-pregnant animals. Even after the injection of supralethal doses of bismuth into the maternal organism, no bismuth could be detected in the fetal liver. From these results, it would appear that the permeability of the placenta for bismuth is only slight. On the other hand, Levy and Selter (87) report that six days after the intramuscular injection of 552 mgm. of bismuth in the form of spriobismol into a rabbit, the fetus contained 0.00297 and 0.00388 mgm. per gram of tissue; the placenta contained 0.00643 mgm. per gram of tissue. Kraul and Bodmer (59) found bismuth in human amniotic liquid after bismuth treatment of the mother, so that the placenta must be permeable even to therapeutic doses. The absence of bismuth from the fetus in Leonard's

TABLE 14

Excretion of bismuth in bile

AUTHOR	PREPARATION	DOSE	TIME	BILE	BLOOD	ANIMAL
		mgm. Bi				
Leonard	Sod. Bismuth Citrate	206	24 hours	0.056	0.0073	Rabbit
		206	24 hours	0.025	0.0018	
		215	24 hours	0.0102	0.0022	
Leonard	Sod. Pot. Bismuth Tartrate	238	24 hours	0.0097	0.0011	Rabbit
		222	24 hours	0.0598	0.0059	
		222	24 hours	—	—	
Lomholt	Bismuth Oxy Chloride in water	5	9 days	0.0016	—	Guinea pig
		8	10 days	0.00006	0.0003	
	Bismuth Oxy Chloride in oil	5	6 days	—	0.0003	
		4	10 days	0.00176	—	

experiments after the injection of single sublethal doses, is, therefore, questionable, and is probably due to analytical defects occasioned by the small quantities of the fetal material. It would be very interesting to compare the bismuth content of maternal and fetal tissue in larger animals, after therapeutic doses.

BISMUTH CONTENT OF THE BLOOD

The quantities of bismuth circulating in the blood are very small. Autenrieth (5) found 0.005 mgm. per gram of blood six hours after the intravenous injection of neo-wismulen. Sei and Weise (127) reported on bismuth values of 0.0012 to 0.085 mgm. per gram, found in the blood

of rabbits killed one to eight hours after the intravenous administration of bismuth sodium tartrate in doses corresponding to 15 mgm. per kgm. Lomholt (89), after the intramuscular injection of suspension of bismuth iodo quinine and bismuth hydroxide into rabbits, found 0.002 to 0.0032 mgm. bismuth per gram of blood; in guinea pigs, he found after the injection of a suspension of bismuth oxychloride, 0.0003 mgm.; Levy and Selter (87) found 0.001 to 0.004 mgm. of bismuth per gram of blood after the intramuscular injection of spirobismol.

Leonard and Seibert (77) followed, in dogs, the appearance of bismuth in the blood after intramuscular injections of minimal nephrotoxic doses (40 to 50 mgm. of bismuth per kgm.) of dipotassium bismuth tartrate (Abbott).

Table 14 gives the results obtained in these experiments. The maximal concentration of bismuth in the blood ranged from 0.001 to 0.003 mgm. per cc. of blood; and was attained forty-eight hours after the injection. If the site of the injection was massaged, the highest bismuth level appeared at the end of the fourth hour, and was maintained for the following forty-eight hours. After this period, the level slowly decreased, but bismuth remained detectable for some weeks after the injection. From these experiments, it may be concluded that with a single injection, there is a steady stream of bismuth through the organism for a considerable period of time.

The chemical form of the bismuth, and its distribution among the different constituents of the blood is of importance for the understanding of the mechanism of its absorption and distribution in the organism. The very low concentration of bismuth in the blood after therapeutically effective doses compared with its relatively low toxicity for spirochetes in vitro, induced Levaditi (80) to assume that the injected bismuth forms in the organism a new chemical compound of highly spirocheticidal properties, which he considers to be the carrier of the therapeutic action. Levaditi and Howard (85) found the therapeutic action of bismuth very much increased under the influence of organs rich in glutathion; Fournier and his co-workers (40) report on the therapeutic efficiency of these rather problematical compounds. Sei (126), working with water soluble bismuth tartrates found by dialysis experiments, that the bismuth forms a compound with the proteins of the serum thus passing from the crystalloidal into the colloidal state. H. Bauer and E. Strauss (11) found in test-tube experiments that bismuth is bound to the euglobulin fraction of the serum proteins; but in his animal experiments, Lomholt (92) found a uniform distribution of bismuth in all

three fractions of the serum. The earlier workers, Mayer and Steinfeld (104); v. Langhans (66); and Meyer and Baehr (98) thought that phagocytic processes were involved in the absorption and circulation of bismuth. Levaditi, however, who originally had similar conceptions, assumes later, that the leucocytes tend rather to keep the bismuth at the site of the injection. Gruhzit assumes also that phagocytosis plays only a minor, if any part in the absorption of bismuth.

Levaditi, Schoen, Girard and Mannin (82) were the first to investigate in animal experiments the distribution of bismuth between serum and cells. After the intramuscular injection of a large dose of bismuth tartrate into a rabbit, they found the ratio of concentration to be about 10 to 1. After intraperitoneal injections in rats and guinea pigs, Lomholt (92) found that about 78 per cent of the bismuth of the blood was in the serum, and the rest in the cruor, using equal parts of both for his determinations. No definite data are available as regards the distribution of bismuth between the red and white blood cells, but experiments of Lomholt indicate that only very small quantities, if any, are found in the leucocytes, and that the erythrocytes seem to be free from bismuth. This would mean that the behavior of bismuth is entirely different from other metals, as for example, lead, which is carried largely by the erythrocytes, but further data are needed.

Because it had been repeatedly assumed that the lipoid solubility of a bismuth preparation would be an important factor in its absorption, Lomholt also determined the bismuth content in the lipoid fraction of the blood without getting positive results.

DISTRIBUTION OF BISMUTH IN THE BODY FLUIDS

The main part of the bismuth is excreted through the kidneys in the urine, which contains quantities of bismuth depending upon the rate of the absorption and on the time elapsed after the administration. The bismuth content of the urine is higher than the bismuth level of the blood, according to Leonard and Seibert (77), but is always considerably smaller than the bismuth content of the kidney.[1] From this, Leonard concludes that the bismuth stored in the kidney has to undergo certain changes before it can be excreted with the urine; but it is conceivable that bismuth, as well as other metals, such as mercury, are merely precipitated in the renal cells by the alkalinity, which must exist in them during the excretion of acid urine.

[1] Similar results have been reported recently by C. Levaditi, Y. Mannin, A. Howard (Compt. rend. soc. biol., cii, 813, 1929).

The presence of bismuth in the bile has been demonstrated by Wichert, Balzer (7) Démelin (34); Lomholt (89) and Leonard (75).

Table 14 gives the results of the two latter authors, Lomholt and Leonards, and shows that with the water soluble compounds the quantities of bismuth excreted in the bile are distinctly higher than those found in the blood, often ten times or more. From this, it appears that there is an active excretion of bismuth in the bile.

It has been shown by Fabre and Picon (38); Levy and Selter (87); and previously also by Dalché and Villejean (30), that the salivary glands contain small quantities of bismuth. As early as 1870, Dembinsky found bismuth in the saliva, and more recently P. Aubry (4) showed that bismuth appeared in the saliva within twenty-two hours after the injection of soluble bismuth citrate and within twenty-four hours after the injection of insoluble bismuth compounds, Trépol and Quinby. Jeanselme and his co-workers demonstrated the presence of bismuth in the saliva of two patients, two and twenty-four hours after the injection of luatol (sodium potassium bismuth tartrate); these findings were confirmed by De Bella (13), Fournier, Cébrian (22) and others.

After the administration of pilocarpine, Démelin (34) has demonstrated the presence of bismuth in the sweat and in the tears of patients under bismuth treatment.

Much attention has been paid to the excretion of bismuth with the milk, because the excretion of antiluetic drugs in the maternal milk is of great importance for the treatment of congenital syphilis. Lewald (88), as early as 1887, reported the appearance of bismuth in the milk of goats, thirty-six hours after the administration of 1 gram of bismuth subnitrate; but the investigations of Cajal, Spierer and Popesco (20) with trépol; of Mme. Cabouat (18) with trépol, néotrépol and quinby gave negative results. More recently, however, Mueller, Blass and Kratzeisen (110), De Bella (13), Bentivoglio (14), and Levy and Selter (87) report positive findings with improved methods. The excretion of bismuth through the milk may therefore be considered as established.

The passage of bismuth into the cerebrospinal fluid has been affirmed and denied, depending perhaps somewhat on the delicacy of the methods. The results of De Bella (13), H. Mueller (108), Mehrtens, Hanzlik, Marshall and Brown (101), and others seem to indicate the presence of bismuth in the cerebrospinal fluid. Von Kennel (54) reported that in febrile conditions more bismuth is present in the liquor than at normal body temperature, but D. Olmer, Arnoux and Massot (119) Christoni (23), (24) and others report negative results.

TABLE 15

Retention of bismuth in the organism

(After intramuscular injection of bismuth oxychloride)

	GUINEA PIG I	GUINEA PIG II	GUINEA PIG III	GUINEA PIG IV	GUINEA PIG V	GUINEA PIG VI
Duration	10 days	9 days	10 days	6 days	17 days	17 days
Total amount of Bismuth injected in mgm	8 in water	5 in water	4 in oil	5 in oil	3 in oil	2 in oil
Animal weight in grams	670	500	500	500	420	470
Total amount of Bismuth recovered in per cent	97.68	90.99	94.27	95.57	80.08	87.20
Radio activity per mgm	4.700	6.400	6.000	700	700	700

Rate of absorption

	ORGAN WEIGHT	μG. Bi	ORGAN WEIGHT	μG. Bi	ORGAN WEIGHT	μG. Bi	ORGAN WEIGHT	μG. Bi	ORGAN WEIGHT	μG. Bi	ORGAN WEIGHT	μG. Bi
Left hind leg	15.2	1.545	—	650	16.3	3133	11.8	1.714	—	71.71	—	68.43
Right hind leg	15.5	2.460	—	1800	17.0	7600	13.1	1785	—	186.7	—	73.14
Total Bismuth at site of injection	—	4.006	—	2450	—	1.073	—	3500	—	258.4	—	141.57
Unabsorbed Bismuth in per cent of injected Bismuth	—	50.08	—	49	—	26.83	—	70	—	8.62	—	7.08

Rate of retention

ORGAN	μG. Bi	MGM. PER CENT GRAM	μG. Bi	MGM. PER CENT GRAM	μG. Bi	MGM. PER CENT GRAM	μG. Bi	MGM. PER CENT GRAM	μG. Bi	MGM. PER CENT GRAM	μG Bi	MGM. PER CENT GRAM
Heart	0.83	0.031	6.25	0.391	1.75	0.103	2.14	0.165	1.57	0.079	0.43	0.024
Lung	6.38	0.152	3.91	0.081	6.97	0.183	1.28	0.027	3.14	0.081	1.86	0.049
Liver	28.55	0.161	34.06	0.383	36.67	0.272	28.57	0.272	—	—	—	—
Bile	0.06	0.006	0.31	0.16	—	—	1.50	0.176	—	—	—	—
Spleen	0.85	0.213	0.94	0.47	1.08	0.240	1.36	0.389	—	—	—	—
Left Kidney	53.83	2.991	27.66	1.257	38.33	2.396	76.00	3.707	—	—	—	—
Right Kidney	47.02	2.766	23.91	1.196	43.00	2.529	65.28	3.184	—	—	—	—
Testicles	—	—	0.63	0.021	—	—	2.83	0.172	—	—	—	—
Small Intestine	16.55	—	1.88	0.020	25.67	0.427	7.43	0.084	—	—	—	—
Large Intestine	122.76	—	132.19	0.796	296.64	1.128	97.86	0.851	—	—	—	—
Brain	0.72	0.018	2.97	0.085	0.70	0.017	3.57	0.102	—	—	—	—
Blood Serum	2.55	0.020	—	—	1.78	0.025	—	—	—	—	—	—
Blood Cruor	0.55	0.010	—	—	0.17	0.004	—	—	—	—	—	—
Bone	3.92	0.047	—	—	16.00	0.178	13.71	0.098	—	—	—	—
Muscle	70.0	0.013	—	—	11.97	0.016	5.57	0.037	—	—	—	—
Skin	22.23	0.034	13.75	0.031	14.88	0.030	(103.57)	(0.188)	—	—	—	—
Rest of the Body	60.94	0.031	68.91	—	28.67		75.57	0.071	166.29	0.054	54.29	0.013
Total Retention in mgm.	374.74		317.17		524.31		486.24		171.00		56.57	
Total Retention in per cent of injected Bismuth	4.68		6.34		13.11		9.72		5.7		2.83	

Total elimination in feces and urine

	URINE (9 DAYS)	FECES (8 DAYS)	URINE (9 DAYS)	FECES (9 DAYS)	URINE (10 DAYS)	FECES (1 DAY)	URINE (6 DAYS)	FECES (6 DAYS)	URINE (17 DAYS)	FECES (17 DAYS)	URINE (17 DAYS)	FECES (17 DAYS)
Mgm. Bismuth excreted	2.407	0.958	1.253	529	1848	324	497	284	1.435	560	1.216	329
Total Excretion	3.433		1.782		2.173		782		1.994		1.545	
Total Excretion in per cent of injected Bismuth	42.92		35.65		54.33		15.65		66.50		77.29	

RETENTION OF BISMUTH IN THE ORGANISM

In discussing the retention of bismuth in the organism, deposits formed at the site of the injection and their retention in the different organs should be distinguished. It is assumed generally that the deposits formed at the site of the injection represent a source from which a continuous stream of bismuth enters the organism, and that the excretion depends on this mobilization. This assumption would justify the study of the absorption from the site of the injection, and of the excretion, in order to determine how much time has to elapse before another dose of bismuth can be administered without bringing about the danger of accumulation. Gruhzit, Tendick and Sultzaberger (46) found that after the injection of bismuth salicylate in oil, the absorption was almost completed at the end of the third week. The elimination, however, did not keep step with the absorption; but was rather low, as much as 50 per cent of the bismuth being retained in the organism, in the course of six months. They believe that the bismuth continues to enter the circulation from its deposits in the various organs, rather than from the site of the injection, and since the organ bismuth is excreted more slowly, neither the study of the absorption, nor that of the excretion would protect against the administration of accumulative doses. For this reason, Lomholt (93) recently performed some experiments for the purpose of checking the results reported by Gruhzit and his co-workers.

Lomholt injected intramuscularly both aqueous and oily suspensions of bismuth oxychloride in doses of from 2 to 8 mgm. of bismuth into guinea pigs, and after six to seventeen days, he determined electroscopically the bismuth at the site of the injection, the bismuth stored in the organism, and the bismuth excreted with feces and urine. Table 15 gives the results of this study; namely, the per cent of the unabsorbed bismuth at the site of the injection, the per cents retained in the rest of the organism, and the percentage eliminated with urine and with feces. From these data it would appear that the portion retained in the organs represents only a small fraction of the bismuth administered. However, it is questionable whether this is typical for other doses and other animals. The percentage excreted is certainly quite different from that reported by other workers for related preparations. While the elimination is reported by Lomholt as 15.65 to 77.29 per cent in six to seventeen days; the dogs of Gruhzit, Tendick and Sultzaberger eliminated only 11.8 to 23.4 per cent in twenty-four to fifty-four days after

the injection of bismuth salicylate suspended in oil; and in man, Delrue found a total excretion of only 6.85 to 11.23 per cent, fourteen to thirty-two days after the administration of the same preparation.

FACTORS INFLUENCING THE ABSORPTION OF BISMUTH

The solubility of the preparation is the most important factor influencing the absorption of bismuth from the deposits at the site of the injection. The best absorption occurs with water and tissue soluble preparations; next range the water soluble preparations which spread over a large surface before they are precipitated by the tissue juices, and with the insoluble preparations, the absorption is distinctly delayed.

Another factor, of importance for the rate of absorption as well as for the production of pain, is the size of the particles or the degree of their dispersion. The finer the particles, the larger is the surface, consequently the smaller particles make more efficient contact with the tissue. Accordingly, Lacapère (64) found for colloidal metallic bismuth, that the therapeutic efficiency runs parallel with the degree of the dispersion, the smallest dispersion having the highest efficiency. Lomholt (94) demonstrated that there is also some relation between the size of the particles and the production of pain. He found that a suspension of particles of from 3 to 4 micra diameter caused no pain, those of 2 micra diameter caused tolerable pain, and very fine particles of from 0.5 to 1 micron produced marked pain. This may be explained by differences in mechanical trauma, due to the greater viscosity of the fine suspensions as well as to their more rapid solution.

The velocity of the absorption is also influenced by the vehicle used in preparing the solution or the suspension. Generally the absorption of aqueous suspensions is more rapid than of the oil suspension of the same preparation, while suspensions in paraffin oil are scarcely absorbed at all.

In this connection, it should also be recalled that bismuth compounds may react with the vehicle, for example, bismuth hydroxide with oil, carbohydrates, and with glycerol.

A further factor in the velocity of the absorption is the concentration of the solution injected. Concentrated solutions may cause a very marked, although localized necrosis of the injected tissue, which is unfavorable to rapid absorption.

Finally, the site of the injection is of great importance, for it has been shown that the absorption occurs faster in muscle tissue, which is actively used, for example, the buttocks, whereas in the resting muscu-

latur, for instance, the musculature of the lumbar region, the absorption is distinctly impaired. For this reason, massage of the site of the injection has been advocated, after the administration, in order to increase the velocity of the absorption.

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THE RÔLE OF HORMONES IN DIGESTION

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On considering the subject of the rôle of hormones in digestion, the question of terminology presents itself. According to Starling (1), the originator of the term, *hormones* are chemical messengers by which one organ exercises a chemical influence on another, or are "the chemical means of correlation of the activities of different parts of the body. Their action may be either the increase or diminution of function, or the alteration of nutrition or rate of growth." The term, however, is rarely used in such a general sense, it being restricted to designate rather specific organic substances formed by certain groups of cells which possess more or less specific physiological properties. The terminology of Schafer (2) is inclusive, etymologically definite, and meets the requirements of our problem. He uses the term "autacoid" (self remedy) as a general term to designate a specific organic substance formed by the cells of one tissue and passed into the circulating fluids producing effects on other tissue functions similar to those produced by drugs. A hormone (I excite), then, is primarily an excitatory autacoid, and a chalone (I make slack) is primarily an inhibitory autacoid; and the effect of an autacoid on a process or function may be hormonic or chalonic.

In this article the term *hormone* will be used in its restricted and etymological sense, and the term *chalone* (2) will be used to designate a specific organic substance formed by a certain group of cells which has an inhibitory action on the functioning of an organ. The term *humoral mechanism*, or *humoral factors* will be used to designate that some change in the chemical composition of the blood has occurred which is responsible for changing the functioning of the organ and when it is not certain what the character of the chemical change is. The term *secretagogue*, which is not infrequently used synonomously with the term hormone, will be used to designate those substances present in food or produced by the digestion or decomposition of food which excite the

secretion of digestive juice either by acting locally, or by being absorbed into the blood or lymph or by causing a hormone to be formed. The terms *gastrin* and *secretin* when unqualified will be used in a specific sense. But when speaking of various tissue extracts, which on parenteral injection excite gastric or pancreatic secretion, the terms *gastric secretin* and *pancreatic secretin* will be used to refer to the active principle. The term *vaso-dilatin* or *dilatin* will be used to designate non-specific substances which cause on parenteral injection a fall in blood pressure, or vaso-dilatation. The term *vaso-depressin* is used by some writers in the same sense as *vaso-dilatin*.

In this review we will consider the effect of the special gastro-intestinal hormones such as gastrin, secretin, etc., and the effect of hormones in general on the secretion and motility of the gastro-intestinal tract.

SALIVARY GLANDS. There is no evidence indicating the existence of a specific hormone for salivary secretion. Variations in the rate of formation and in the quality of saliva are explained entirely by nervous influences affecting the secretory cells, the blood flow, and the contractile elements of the gland (3). Recently Sacks and Kim (4) have extracted the buccal, pharyngeal and lingual mucosa with acid and have found that such extracts do not contain a substance which on injection will cause a secretion of saliva and have confirmed Langley (5) and Malloizel (6) by finding that the recently denervated submaxillary gland will not secrete when acid is applied to the mouth.

The effect of extracts of a number of glandular tissues on salivary secretion has been studied, such as secretin, gastrin, epinephrine, pituitrin, insulin, thyroiodin, and such substances as histamine and choline, which are known to be present in some tissue extracts.

According to Deroux, Lambert and Myer (7) and Starling (8), secretin prepared from the duodenal mucosa has no effect on salivary secretion. Gastrin prepared from the pyloric mucosa and given in (physiologic) doses sufficient to cause marked gastric secretion has never in the author's experience of several hundred subcutaneous injections caused a salivary response. Whether secretin or gastrin have any effect on sensitizing, depressing, or modifying the secretory mechanism is unknown. The action of histamine, which is related to gastrin, has been studied recently by MacKay (9), who found that it causes a slight increase in the spontaneous secretion due to an action on the secretory cells and on the contractile elements of the gland. In her experiments, the histamine was administered intravenously in doses of from 0.25 to 2 mgm. within a short period of time, which means that the

doses were not within a physiologically possible range, and explains the absence of salivation, unless nausea is provoked, in human subjects and dogs when 1.0 mgm. of this drug is given subcutaneously. Since it was observed in her experiments that the secretory effect of histamine was augmented by previous stimulation of the chorda tympani nerve, it should be determined whether or not purified preparations of secretin, but more particularly gastrin, have a similar action.

A dose of epinephrine larger than that required to raise blood pressure causes the submaxillary gland to secrete (10) (11) in the dog and cat, the increase being more marked in the cat because of the well known difference in the functional innervation of the gland in these two animals. Florovsky (12) has found that the denervated salivary gland of the cat is particularly sensitive to the action of epinephrine and that such a gland would not be excited to secrete on stimulation of the sciatic nerve, if the adrenals had been previously extirpated.

Pituitrin (13) in pharmacologic doses depresses the secretion of saliva and even reduces the pilocarpine response.

Insulin (14) in non-toxic doses has no effect on the salivary glands.

Thyroiodin, according to Krontovsky and Maevsky (15) and Poinrovsky (16), sensitizes the secretory nerves of the salivary glands. Their opinion as to the portion of the secretory innervation affected differs because the former used cats and the latter used dogs. In the dogs, the salivary response to epinephrine and reflex excitation was increased after thyroiodin. In cats, the pilocarpine secretion was increased, whereas this does not occur in dogs.

Choline is a well known parasympathetic excitant and certain symptoms classed as "vagotonic" (salivation, bradycardia, etc.) have been ascribed to abnormal amounts of choline in the blood. Choline has been found in the various body fluids and in tissues that have undergone some autolysis (17) (18). It has even been postulated by Le Heux (19) that choline is a gastro-intestinal motor hormone. Certain of its chemical derivatives are very potent (20) (21). Hunt (17), however, found no marked increase of choline in the blood serum in a number of pathological conditions, and that on intravenous injection it disappears from the blood with great rapidity. The presence of choline in saliva and its relation to salivary secretion will be referred to later.

Summarizing our knowledge of the action of tissue extracts, or of pharmacological substances present in the body tissues, on the salivary glands, it can be stated that there is no direct evidence showing that any of these substances plays a rôle in normal salivary secretion.

It is generally stated by physiologists that the by-products of tissue activity not only increase the blood flow to the active tissue, but also increase the response of the tissue to stimuli; an example of the former phenomenon i that the increase in blood flow of the submaxillary gland o chorda stimulation is greater before the administration of atropine than after it, and an example of the latter phenomenon is the fact that if the gastric glands are secreting some free acid, they are more readily stimulated than if they are not secreting free acid. The interpretation of this phenomenon is not at all clear, because it is not certain whether the phenomenon is in part or entirely due to the by-products, and if so it has not been shown that the by-products are in any way specific.

Claude Bernard (22) injected saliva intravenously into dogs with negative results. Schafer and Oliver (23) injected extracts of the salivary glands and observed nothing but a fall in blood pressure which is characteristic of so many tissue extracts.

Demoor (24) has done some work bearing on this problem. He perfused the submaxillary gland of the dog *in situ* with Locke's solution plus blood serum and found that on adding an extract of the resting gland no secretion occurred, but that on adding an extract of an active gland or one that had been secreting, a secretion resulted. He attributed the stimulation to substances in the saliva which he found had a similar effect. He found that these substances prolonged the duration of the chorda tympani response and were destroyed by heating to 50° to 65°C. Demoor stated that it was necessary to add blood serum or defibrinated blood to the perfusing solution in order to obtain a secretion of the perfused gland on chorda stimulation. Quagliariello (25), however, found the blood serum to be unnecessary. Since there is choline in saliva and in blood serum (17) it is possible that the effects observed by Demoor are due to this substance (26), especially since choline is decomposed by heat. Obviously more work of the nature of Demoor's and Quagliariello's and of a more direct physiological character must be done before we will know whether by-products of glandular activity act in a specific manner. We should know such things as the choline content of the resting and active gland, of the gland and saliva during paralytic secretion, and as the effect of salivary gland lymph and venous blood on the nervous and secretory elements of the salivary glands.

A number of investigators have attempted to ascertain whether or not the salivary glands produce an internal secretion which affects other glandular tissues.

Fehr (27) was the first to attempt extirpation of the salivary glands. He found that this procedure had no appreciable effect except that the animal drank more water. Schafer and Moore (28) observed a similar result. Simonetta (29) has quite recently attempted extirpation of the glands in rabbits with negative effects.

Romer (30) has reviewed the literature on the alleged internal secretory relationship between the salivary glands, pancreas and gonads. Such a relationship is based on the clinical fact that parotitis is in some way associated with pancreatitis and orchitis. Romer came to the conclusion that there is no established endocrine inter-relationship between these organs. However, there is considerable modern literature on the inter-relationship of the salivary gland and pancreas in relation to blood diastase and sugar metabolism which will not be reviewed because it is only indirectly relevant to the subject at hand (31).

Hemmeter (32), studying the effect of salivary gland extirpation on gastric digestion in the dog, came to the conclusion that a hormone is produced by the salivary glands which controls normal gastric secretion. He reported that extirpation of the salivary glands in gastric fistula dogs caused diminished gastric secretion and peptic digestion although not marked in some cases, which could be returned to normal by injecting or feeding extracts of the salivary glands. Loevenhart and Hooker (33) injected intravenously extracts of salivary glands into gastric fistula dogs with negative results on gastric secretion. Hemmeter (34) pointed out that these results on "normal" dogs with salivary glands did not necessarily disagree with his results. Keeton and Koch (35) were unable to extract gastrin from the submaxillary glands. The work of Hemmeter has been repeated by Swanson (36) who used Pavlov pouch dogs. He found that removal of the salivary glands did not alter the rate or quantity of the secretion, the average acidity being slightly increased. Swanson's results certainly show, along with the results of Keeton and Koch, that the salivary glands do not produce a hormone for gastric secretion.

The clinical sialorrheas and oligosialias, in the light of our present knowledge, are all best explained on a reflex basis, or by the action of toxins on the salivary centers, nerve endings, or gland cells, or by changes in the gland itself.

GASTRIC GLANDS. Prior to the introduction of the gastrin theory by Edkins in 1905 (37), the excitation of the gastric glands by food in the stomach was explained either by mechanical stimulation, or by the action of secretagogues in the food on a local nervous secretory mecha-

nism, or by the absorption into the blood of secretagogues present in the food or produced by its digestion. Although one gathers from Beaumont's studies (38) that he had a vague concept of chemical excitation of the gastric glands, the first definite expression of this concept came from Blondlot (39). Heidenhain (40) was aware that various foods might have a different effect on the gastric glands. Lobasov (41), working in Pavlov's laboratory and employing dogs with a gastric fistula and a Pavlov pouch, observed that the introduction of certain substances into the stomach caused a secretion in the pouch after a latent period of thirty minutes. Although open to criticism (42), this was the first experimental attempt to demonstrate local chemical stimulation. Khizhin (43) demonstrated that the gastric digestion of certain food substances increased their potency as gastric secretory excitants when introduced into the stomach, a fact which has been confirmed by Farrell (44), using methods not open to criticism. Sokolov (45), Lonnquist (46) and Pavlov (47) employed animals with a Pavlov pouch, a gastric fistula, a duodenal fistula, and the stomach separated from the duodenum by either incising the pyloric sphincter or by making a septum with mucous membrane, and found that some substances caused secretion when placed in the stomach and others when placed in the intestine. The view that was first offered by Pavlov and his colleagues to explain this stimulation was that the secretagogues excited a long reflex through the cerebro-spinal nervous system. However, when Popielski (48) showed that stimulation did not occur on intravenous injection of meat extract, and did occur when meat extract was placed into the stomach of dogs with vagi and splanchnics cut and coeliac plexus removed, the explanation that the stimulation was due to a local reflex mechanism in the stomach wall was adopted.

Shortly after the discovery by Bayliss and Starling (49) that extracts of the duodenal mucosa would excite pancreatic secretion, Edkins (37), using objectionable methods, discovered that extracts of the pyloric mucosa on intravenous injection caused the gastric glands to secrete. He called the substance "gastric secretin" or gastrin. Gross (50), working in Pavlov's laboratory about the same time (1906) performed experiments which suggested a pyloric hormone mechanism. He used a preparation similar to that of Sokolov with the exception that a septum, or wall of mucous membrane, separated the fundus from the pylorus, so that on introducing meat extract, etc., into the duodenal fistula it could pass back to the pylorus, a method which did not rule out intestinal stimulation. Because Sokolov and Lobasov had ob-

tained positive results with meat extract in the entire stomach and negative results in the intestine, he concluded that his positive results were due to the action of the meat extract on the pyloric antrum. Krzyszkovski (51) and Orbelli (52), working contemporaneously with Gross, arrived at the same conclusion. Orbelli, however, still held that a part of the pyloric secretagogue effect was due to nervous connections because the response of the Pavlov pouch decreases so markedly after section of its nerves. Edkins and Tweedy (53) devised a balloon method which enabled them to separate functionally the pyloric from the fundic end of the stomach and to study the effect of substances in the pyloric antrum on the secretion of the fundus. They obtained evidence of stimulation, which, however, has been challenged (54).

These experiments led to a general acceptance of the gastrin theory, and stimulated numerous investigators to a further analysis of the theory.

Popielski (55) found a large group of substances which caused on injection a fall in blood pressure as well as gastric secretion. He came to the conclusion that the activity of the Edkins pyloric extract was due to vaso-dilatation and not to the specific effect of a hormone. Numerous investigators confirmed Popielski (56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66), finding that a "gastric secretin" could be extracted from many animal tissues (Witte's peptone, fundic pyloric and intestinal mucosa, thyroid, brain and pancreas) and even plants.

Luckhardt, Koch and Keeton (59) found that gastrin could not be extracted from gastric juice and Ivy and Oyama (67) could extract none from gastric mucous, although Eisenhardt (63) states that he provoked a secretion by injecting the mucous secretion from the pyloric region but not the fundic region. Maydell (68), however, was able to obtain an active extract from pyloric mucosa, fundic and duodenal mucosa only, gastric juice being inactive.

Zeliony (69) and Savitsch and Zeliony (70) made a definite contribution by using animals with an isolated pyloric pouch and a gastric fistula. They observed that application of certain substances to the pyloric mucosa stimulated gastric secretion, which was prevented from occurring if atropine had been previously administered or if the mucosa of the pyloric pouch had been treated with cocaine. These observations led these observers to conclude like Orbelli that a nervous mechanism was concerned in the stimulation they observed. (To be discussed more fully later.) Rheinboldt (71) in Bickel's laboratory observed secretion in an extrinsically denervated Heidenhain pouch (a Bickel pouch),

which has been confirmed by others (72), a fact that supports a humoral mechanism. Ivy and Whitlow (54) repeated the experiments of Savitsch and Zeliony with the exception that they used a Pavlov pouch instead of a gastric fistula. They observed no excitation of the fundic pouch when various substances were applied to the pyloric pouch. Cinnimata (73) performed experiments analogous to those of Ivy and Whitlow with positive results soon after the operation, but with negative results at a later period. He obtained without failure positive results when he used a gastric fistula instead of a Pavlov pouch preparation. Lim and Ivy and McCarthy (42) have also observed that distention and stimulation of the isolated pyloric pouch would increase the secretion in gastric fistula dogs. The author explains the discrepancy between the results of Ivy and Whitlow and those just cited by assuming that the Pavlov pouches used by them were more refractory than the stomach proper, even though it was shown that the pouches responded to a meal.

The pharmacodynamic method of making tissue extracts, injecting drugs, amines, etc., demonstrated that a number of substances could excite the gastric glands, but did not materially assist in settling the question of a gastric hormone. The making of various types of pouches and fistulae, the application of food substances to various portions of the gastro-intestinal canal, with and without sectioning certain nerves taught us much concerning the physiology of gastric secretion, but did not clarify the hormone situation. In 1921, three methods were still available for use in the study of this question: the method of blood transfusion, the method of cross-circulation, and the method of transplantation.

Lim (74) in 1922 reported observations in which he transfused and cross-circulated unfed with fed animals (cats) with negative results except in two doubtful cases. In these experiments the cross-circulation time was too short to be satisfactory and the experiments were done under anesthesia which might mask or prevent any possible positive result. Ivy, Lim and McCarthy (75) repeated the work of Lim on unanesthetized dogs. After showing that the bloods of the donor and recipient were compatible, they transfused from 150 to 200 cc. of whole blood by the Percy-Brown-Kimpton tube technique, using only Pavlov pouch dogs and taking "fed" blood at the height of secretion as indicated by the pouch secretion. They came to the conclusion that blood transfusion, even under the best of conditions is an inadequate procedure with which to put to test the possibility of either a hormone or a

humoral factor in the genesis of gastric secretion. Their results showed that when gastrin or histamine was injected there was not enough of it in the blood at any moment to cause a definite gastric response which made it appear that substances which excite gastric secretion do so by a cumulative action. They also obtained some positive responses when they ran a "control experiment" of transfusing "unfed blood" to an "unfed animal," or in other words the mere increase in blood volume caused a slight response in some experiments. Rasenkov (76) about the same time published observations on the effect of the transfusion of "fed blood" with results which he interpreted as being positive. He transfused from 170 to 200 cc. of blood, but defibrinated it before transfusing it, and failed to run adequate controls. The responses he observed were quite small except in one experiment. Ivy, Lim and McCarthy (75) performed three cross-circulation experiments in Pavlov pouch dogs that responded well to a meal and whose bloods were compatible on the basis of agglutination tests, the animals being allowed to recover from the anesthetic and held firmly together with a cast as long as 72 hours. On feeding a meal to one of the pair, they observed suggestive but not decisive increases in gastric secretion and expressed doubt that a cross-circulation experiment is to be considered as a crucial experiment for the gastric hormone problem, because less than one-sixth of an injected drug passes from the injected animal to the uninjected animal. Ivy and Farrell (77) auto-transplanted small pouches of the fundic portion of the stomach subcutaneously in dogs and observed that following a meal the transplant secreted acid. The latent period of response was one hour or more which they showed was due to the refractoriness of the pouch caused by its decreased blood supply. Lim, Loo and Liu (78) confirmed the foregoing observation by transplanting a fundic pouch, but using a different technique. These workers made the transplant by using the method of direct blood vessel anastomosis. On feeding the animal, stimulation of secretion of the transplant was observed after a latent period of forty minutes. They observed, as did Ivy and Farrell that mechanical distention of the pouch sometimes provoked a response. Further, Lim and Necheles (79) have shown by vivi-dialysis of the blood of a fed dog that a gastric secretory excitant can be obtained from the blood of the portal and systemic circulation.

The results on transplantation and vivi-dialysis prove and establish a humoral mechanism for gastric secretion

This is the story, in brief review, of a thirty year attempt to understand the mechanism of chemical excitation of the gastric glands on the

ingestion of food. Although the attempts have resulted in establishing a humoral mechanism for gastric secretion, they do not prove whether the humoral agent is secretagogic or hormonic (gastrin). Before this question can be satisfactorily answered the chemical composition of gastrin must be determined and shown to be identical with the excitant isolated from the blood by vivi-dialysis.

An analysis of the evidence bearing on the question of whether the humoral agent is hormonic or secretagogic will not be undertaken. However, it should be pointed out that mechanical or "pure" chemical stimulation (69) (70) (80) of a "denervated" pyloric pouch will cause a stimulation of the fundic glands, and mechanical or chemical stimulation of the stomach will cause secretion in the "denervated" fundic pouch (42). The most logical interpretation of such results in view of the fact that a gastric secretory excitant can be isolated from the pyloric mucosa, which is not histamine (to be discussed later), is that the secretion is due to a hormone.

The regulation of hormone production. Granted that there is a hormone mechanism, the question logically arises, what controls its production and elimination into the blood stream. Savitsch (80) explained the hypersecretion of gastric juice which he observed following the separation by incision of the pyloric antrum from the fundus by assuming that the vagus nerve controls the passage of the pyloric hormone into the blood stream. Zeliony and Savitsch (70) (81) found that an injection of atropine or the application of cocaine to the mucosa of a pyloric pouch, prevented gastric stimulation when the pyloric antrum was excited by the application of meat extract. They believed the atropine effect was due to paralysis of the efferent side of the reflex arc and the cocaine effect was due to paralysis of the afferent side. That the cocaine did not act by causing vaso-constriction and preventing the absorption of the hypothetical gastrin was shown by the use of epinephrine which did not prevent stimulation when the meat extract was applied. In this connection it is well known that relatively large doses of atropine are required to prevent the action of gastrin (82) on subcutaneous injection, whereas a small dose of atropine prevents gastric secretion to a meal in the dog. The paradoxical nature of these two latter facts has been an argument against the gastrin theory. This discrepancy as well as the observations of Zeliony and Savitsch can be explained by assuming that atropine and the local application of cocaine prevents the gastrin from being formed but after gastrin is once formed (as in extracts) atropine will not prevent it from acting. In this con-

nection it should be pointed out that Lim, Ivy and McCarthy (42) found that atropine prevented the stimulation resulting from mechanical distention of the stomach. It also prevents the secretion of a "denervated" fundic pouch, but has not been tried on the transplanted pouch. It should also be kept in mind that in the above studies, impure gastrin was used.

How does the humoral agent act? How the humoral agent acts to bring about gastric secretion is not known. A number of opinions have been expressed. Bickel (83) holds that the nervous system regulates the gastric secretory cells, and that when the cells are freed from the nervous system they react with chemical stimuli in the blood. Krimberg (84) has expressed a similar view to the effect that the nerves do not permit the hormone to enter the cells during rest, but during activity the nerves lose this influence. He thinks that the hormone passes out through the cells into the secretion, although the evidence is against the presence of gastrin in gastric juice. It is certain that impure gastrin and atropine affect the secretory mechanism at different points (82); but in toxic doses, the atropine effect spreads over to the point of the mechanism affected by gastrin. Since gastrin, even in a "purified" state (85), and the closely related compound histamine have a vasodilator action, it has been postulated that these substances stimulate gastric secretion by increasing the blood flow through the gastric glands, and Lim, Ivy and McCarthy (42) have suggested this as a possibility for explaining augmentation of gastric secretion on mechanical distention. Lim, Necheles and Ni (86) and Ni and Lim (87) studying the vasomotor reactions and metabolism of the vivi-perfused stomach came to the conclusion that an increase in blood flow is not an essential accompaniment of secretion. The vivi-perfused stomach can secrete to histamine while the gastric blood flow is steadily diminishing. This does not mean of course that increased blood flow does not occur during normal secretion. Gastrin, histamine and the ingestion of a meal increase the permeability of the gastric cell membrane for substances such as dyes (88) which are not normal constituents of the gastric juice; but how this change in permeability is brought about is no more understood in the case of the gastric secretory cell than for any other cell. The evidence indicates that both gastrin and histamine act through some cumulative action, since it is necessary to maintain in the blood a certain concentration of these agents for relatively a long period of time (ten to fifteen minutes) and since there is not a sufficient quantity in the blood at any moment to excite the gastric glands (75) (89).

How do secretagogues act? It is definitely established that some foods (meat) in the raw state possess the property of exciting gastric secretion on local contact with the gastric mucosa, the pyloric mucosa being more sensitive in this respect than the fundic (42). It is also true that the digestion of foods, which do not have such a property in the raw state, yields products which do have this property (44). Ivy and Javois (89) (90) (91) administered hydrolyzed proteins, pure amino acids, pure amines and allied substances in an attempt to ascertain the nature of the secretagogues and manner of their action. They found that hydrolyzed proteins act chiefly via the intestine and cause secretion of a denervated fundic pouch, which is prevented by atropine (1 mgm.). Subcutaneously they had no action which confirmed the observations of Schweitzer (92). By mouth hydrolyzed protein was a more potent excitant than the raw protein. They were not given intravenously because of their marked action on blood pressure and the results would not be interpretable. However, several have reported that Liebig's meat extract injected intravenously causes a gastric secretion which is not prevented by atropine (93) (81).

Bickel and Ehrman (57) (94) found that amino acids would stimulate gastric secretion, but Schweitzer (92) pointed out that this was not a common property. Ivy and Javois (91) used twenty pure amino acids in their work. Of the twenty, twelve were active by stomach tube in relatively large doses of from one to two grams, β-alanine being the most active. Atropine prevented a response. None of them was active in the sense of "gastric secretins," because subcutaneously they were ineffectual. An amino acid mixture containing most of the amino acids of casein was not as potent an excitant as casein hydrolyzed by acid or "pancreatin," which they explained by assuming that hydrolyzed casein contains other substances which are more potent gastric secretory excitants than the amino acids. The latent period of action of the amino acids was one hour or more which led them to believe that their action was due possibly to the amine or aporrhegma of the amino acid being formed in the intestine; and that the action of hydrolyzed proteins is due to polypeptides, amino acids and amines working together. They found that a number of amines given by mouth or subcutaneously excited gastric secretion.

Some of the secretagogues are known to act via the stomach (44) (47) (42) (89) (90) (91); others are known to act via the intestine (72) (90) (91) (95) and some, such as histamine and β-alanine and digested proteins (42) (44) (90) (91) (72) act via both stomach and intestine. All these substances have not been tried out at both sites, however.

Other substances than protein products and derivatives excite gastric secretion by acting in the intestine such as soap (95) (96), dilute HCl, 10 per cent glycerin, spinach juice, $MgSO_4$ and saponin (a very slightly absorbable substance) (72). Glucose has a variable action sometimes stimulating and sometimes not (72) (95). The latent period of action is more than thirty minutes.

It is uncertain how the secretagogues act. The amines as a group probably act by being absorbed, since they act on subcutaneous injection and after atropine. The application of histamine to the intestinal mucosa causes stimulation even when cocaine has been previously applied; this is also true of stimulation caused by applying dilute HCl to the intestinal mucosa. That hydrolyzed proteins do not act entirely through a reflex mechanism is shown by the fact that they excite secretion in a "denervated" fundic pouch when given by stomach tube. Ivy and Javois (89) (90) (91) state that they probably do not act by being absorbed because of the negative results of Ivy, Lim and McCarthy (75) on blood transfusion and cross-circulation, and express the opinion that they may act by causing an increase in the blood flow through the entire portal area by exciting vaso-motor reactions. Since it is now established that there is a humoral agency for gastric secretion, secretagogues may act either by causing the hormone (gastrin) to be formed in the stomach or intestine, since it is known that a gastric secretory excitant can be extracted from gastric and intestinal mucosa, or by being absorbed. Since the effect of hydrolyzed protein by mouth is prevented by atropine, and since hydrolyzed protein subcutaneously does not stimulate (89) (90) and the results in the literature on such a procedure are variable (92) and since large amounts of pure amino acids and amines (90) (91) are required to stimulate gastric secretion when given by mouth, etc., the author's opinion is that the evidence supports the explanation that secretagogues present in the food or produced by the digestion of food excite gastric secretion through a hormone mechanism in the normal condition of the gastro-intestinal tract. Rasenkov (76) holds that they act by being absorbed. The only way this question can be settled is to ascertain the chemical properties of the gastrin from pyloric extracts and then show that the gastric secretory excitant in vivi-dialysates is the same substance chemically and that there are no other gastric secretory excitants in the blood during digestion.

Specificity of gastrin. The chemistry of gastrin, or the active principle of gastric and intestinal mucosal extracts, must be thoroughly understood before we are able to answer satisfactorily the question of

the specificity of gastrin. The presence of a gastric excitant (which acts on subcutaneous injection) in meat, digested bread, spinach, etc., does not disprove the existence of a specific hormone, gastrin, since it is known that various compounds, such as histamine and pilocarpine, excite gastric secretion and similar products may be present in any of the extracts made.

Working with crude preparations, Luckhardt, Henn and Palmer (97) found that a "gastrin preparation" would not only stimulate the flow of gastric juice but also the flow of pancreatic juice and a Bayliss and Starling "secretin" preparation would stimulate both the formation of pancreatic and gastric juice, the correctness of which the author has had the occasion to observe a number of times. But this is not true of purified solutions of secretin and gastrin. Weaver (98) has found that purified secretin would not stimulate gastric secretion, which the author and his students have verified. Further, we have found that "purified" gastrin will not cause pancreatic secretion (99). Chemically gastrin and secretin are not at all related. To argue that gastrin is not a specific hormone on the basis of the fact that there is a gastric secretory excitant in many animal and plant tissues is analogous to arguing that insulin is not a specific hormone on the basis of the fact that there is a blood sugar lowering principle in many animal and plant tissues.

Can histamine be the gastric hormone? A question that always comes to the front in a discussion of gastrin is, "Can histamine be the gastric hormone?" Popielski believed that the excitation of gastric secretion on the subcutaneous injection of various extracts was due to one or more substances which he called "vaso-dilatins." Dale and Laidlaw (100) compared vaso-dilatin to histamine and suggested that they might be the same. Popielski (101) investigated histamine and accepted the suggestion because it stimulated gastric secretion and decreased blood pressure. Keeton, Koch and Luckhardt (102) in the same year found that histamine excited gastric secretion, but stated that "The behavior (toxicity and secretory curve) of gastrin does not conform sufficiently to histamine to warrant the conclusion that they are the same substance." Rothlin and Gundlach (103) concluded that histamine and gastrin are identical. Koch, Luckhardt and Keeton (104), after a study of the chemistry of gastrin, came to the conclusion that histamine and gastrin are quite similar, but observed that gastrin was neither precipitated by picric nor by picrolonic acids, whereas histamine added to a gastrin solution was precipitated by these reagents. Ivy and Javois

(89) found that 0.0027 mgm. per kilo per minute of histamine could be injected intravenously in an unanesthetized dog causing gastric secretion without peripheral vaso-dilatation or objective symptoms. According to Koessler and Hanke (105) this is the minimal dose that will cause a fall in blood pressure. Ivy and Javois (89) and others found that histamine by mouth in relatively large doses (225 mgm.) would stimulate gastric secretion without the production of symptoms. It is very questionable whether there are sufficient quantities of histamine in the lumen of the bowel to make this observation physiologically significant. There is, of course, sufficient vaso-dilatin material in the lumen of the intestine to have physiological significance if it were known that it acted similarly to histamine. A milligram of atropine antagonized threshold doses of histamine given subcutaneously (106), but atropine in 1 mgm. doses does not antagonize submaximal doses (82). The same is true for gastrin, but it should be pointed out that the gastrin used was crude, having been purified only by alcohol and basic lead acetate precipitation. Abel and Kuboda (107) were able to isolate from the combined gastric and intestinal mucosa a substance which they believe was histamine picrate and which had the physiological properties of histamine. But histamine has not been isolated from pyloric or gastric mucosa alone. Barger and Dale (108) isolated histamine picrate crystals from the mucosa of the small intestine and thought that histamine was identical with secretin, which we know now is not the case. Sacks and Burgess (109) working in the author's laboratory on fresh (20 minutes after death) hog's pyloric mucosa have verified the chemical findings of Koch, Luckhardt and Keeton (104) on gastrin. The literature shows that there are a number of substances that excite gastric secretion, but unquestionably the substance most closely related to gastrin is histamine. In the light of our present knowledge, we are warranted in stating that histamine is closely related to but not identical with the active principle of pyloric mucosa extracts.

Chemistry of gastrin. All that the literature yields on the subject of the chemistry of gastrin (the active principle of extracts of gastric mucosa) is contained in the article by Koch, Luckhardt and Keeton (104). The work of Sacks and Burgess will be published soon.

Fat inhibition of gastric secretion. The inhibitory action of undigested fat (digested fat such as soap stimulates) was studied by a number of Pavlov's students (47)—Damskin, Lobasov, Sokolov and Orbelli. Fat not only inhibits the response to a meal as long as it remains undigested

but also inhibits the action of the vagus in the cephalic phase of gastric secretion as shown by sham feeding experiments. Sokolov (47) showed that this action was due chiefly to the fat acting in the intestine which has been confirmed by Smidt, (110) and by Lim, Ivy and McCarthy (42). The latter workers also demonstrated that fat had a slight inhibitory action when in the stomach, and that fat in the intestine would inhibit the action of a small dose of histamine, when the latter was applied to the gastric mucosa. Orbelli (111) in his work observed that fat did not inhibit the secretion to an isolated pouch which had been deprived of its vagus innervation. He concluded that fat inhibition was dependent upon a vagus reflex mechanism. Ivy, Lim and McCarthy (72) failed to confirm this finding and found that fat inhibition occurs after "complete denervation" of a Heidenhain pouch (Bickel pouch). Feng, Hou and Lim (Chinese J. Physiol., 1929, iii, 371) have recently found that ingestion of fat causes inhibition of gastric secretion in auto-transplanted gastric pouches which proves the existence of a humoral mechanism for fat inhibition.

Evaluation of the humoral mechanism. It is obviously impossible to evaluate the rôle of a hormone in gastric secretion because it is not absolutely known whether the humoral agent is hormonic or secretagogic. One can in only a general way guess at the relative importance of the humoral mechanism.

There is no evidence that a hormone is produced during the cephalic phase of gastric secretion which might be analogous to the cardiac "vagusstoff" of Loewi (112) (26). In the transplanted fundic pouch, the latent period is longer than the average duration of the sham feeding response, which is also in a general way true of the Heidenhain and Bickel ("denervated") pouch, and from an anatomical viewpoint the post-ganglionic vagal fibers should be intact in these preparations.

In the gastric phase two types of stimuli are acting in the stomach,—mechanical distention and chemical excitants. The mechanism responsible for the excitation of secretion on mechanical distention is not known. The facts are that distention of the main stomach may cause a "denervated" pouch to secrete (42), that distention of the transplanted fundic pouch may cause it to secrete (77) (78) and that the post-ganglionic vagus fibers seem to be necessary. The possible explanations are: 1, an active or favorable increase in the blood flow; 2, a local nervous mechanism; 3, or the elaboration of a hormone. Neither is the mechanism for the excitation of secretion by chemical excitants acting in

the stomach definitely known. The facts are that the pre-ganglionic vagus-fibers are not necessary, that the post-ganglionic vagus fibers seem to be necessary (on the basis of atropine evidence in the dog), that the coeliac ganglion is unnecessary, that cocaine on the pyloric mucosa prevents secretion elicited by stimulation of the pylorus, that absorption from the stomach is much less than it is from the intestine, that gastrin can be extracted from the gastric mucosa, that separation of the pylorus from the fundus decreases the response, that raw fresh meat juice or fresh meat juice brought to the boiling point excites on local application to stomach, and that an extract of fresh skeletal muscle does not cause gastric secretion on subcutaneous injection (intravenous injection too unphysiological). These facts indicate that there are two mechanisms concerned: a nervous (vascular or secretory) and a hormone, and that the formation of the hormone is dependent to a great extent on the integrity of a nervous mechanism susceptible to atropine. (Our studies on the antagonism of atropine and gastrin unfortunately have been made while using crude gastrin, and the literature reveals discrepancies in the effect of atropine on the gastric response to a meal in man.)

The intestinal phase of gastric secretion is due entirely, according to the evidence at hand, to chemical excitants acting in the intestine. The mechanism is not known. The facts are that many substances normally found in the intestine excite gastric secretion when artificially introduced into the intestine, that distention of a small loop (12 in.) of the gut does not excite secretion in a pouch, that the pre-ganglionic vagus fibers are unnecessary for excitation of the gastric glands from the intestine, that atropine prevents excitation, that the coeliac ganglion is not necessary, that enteric connections are not necessary, that a non-absorbable or poorly absorbable substance such as saponin, or $MgSO_4$ may be an effective stimulus, as well as absorbable substances, and finally that a "gastric secretin" can be extracted from the intestinal mucosa, but it is not known that it is identical with gastrin. The facts indicate that the chemical excitants in the intestine act chiefly, if not entirely, by either being absorbed or by causing a hormone to be formed. If they act by being absorbed, then atropine (in the dog in small doses at least) either prevents their absorption or prevents them from acting after being absorbed. Unfortunately we do not know the whole story of the effect of atropine on absorption, and it is not likely from what we know of "gastric secretins" in general that atropine would prevent these chemical excitants from acting after absorption. If the

chemical excitants caused a hormone to be formed, then atropine would act by preventing the hormone from being formed.

We will not be able to evaluate correctly the rôle that secretagogues or a hormone play in digestion until we are able to differentiate chemically between the two and know their state of absorption or production and the intensity of their action. A complete knowledge of the mode of action of atropine on glandular mechanisms would be of material assistance, since at the present time we feel uncertain of any interpretation of results on the effect of atropine on secretory processes. But in spite of our lack of exact knowledge, it is obvious that a humoral mechanism plays an important, if not a very fundamental, rôle in the physiology of gastric secretion.

The rôle that a humoral agency may play in the qualitative and quantitative differences in the gastric secretory response to different foods will not be undertaken, first, because we do not know enough concerning the nature of the humoral agency, and second, because a statistical study is necessary to furnish exact data on the qualitative and quantitative differences in the response to different foods and such a study has not been made. The data provided by Pavlov are insufficient and the qualitative difference in the juice secreted on feeding different foods may be due to differences in secretory rate (113).

According to Dragstedt and Dragstedt (114), a humoral agency may be concerned in the production of fluid by the stomach in "acute dilatation" of the stomach and intestinal obstruction. It may possibly play some rôle in the gastric secretory disturbances observed in certain diseases of the colon (115). Kalk (116) has reported that stimulation of the skin, in patients with dermatographism, causes an increase in gastric secretion, which he attributes to the liberation of a histamine-like substance by the skin in these cases. So many points of this nature are found in the literature that it is impossible to mention them all, and these have been selected to show how widely the idea of a humoral agency has been applied.

Effect of endocrine glands on gastric secretion. Parathyroids: Removal of the parathyroids decreases gastric secretion (117), but if the tetany is controlled gastric secretion is unaffected (118). Parathormone does not influence gastric secretion if the water balance is maintained and if its administration is not carried to the point of toxicity. Overdosage abolishes gastric secretion at the point at which hemorrhage into the gastric mucosa occurs (119).

Thyroid: Disorders of digestion in disease of the thyroid in man are

well known. Boenheim (120) reported gastric hypersecretion in hyperthyroidism. Neilson (121) also reported hyperacidity. Leist (122) and McCaskey (123) reported subacidity and achylia in exophthalmic goiter, but normal secretion in other types of hyperthyroidism. Lockwood (124) found decreased acidity in both hyper- and hypothyroidism. Sturgis (125) reported achlorhydria in three of five cases of myxedema. Katz (126) found hyperacidity in cases of hypothyroidism which were benefited by thyroid feeding. Badylkes (127) found that large doses of thyroid extract causes decreased secretion and small doses increased secretion. Lewit (128) noted increased gastric secretion on administration of thyroid extract in man. He states that patients with hyperthyroidism have as a rule hyperacidity, whereas hypoacidity or achylia is frequent in exophthalmic goiter. Hardt (129) and Truesdell (130) observed decreased gastric secretion on feeding toxic doses of thyroid extract to Pavlov pouch dogs.

The clinical observations are conflicting, but are explained in part by the results of Lewit and Badylkes. The experimental reports of feeding thyroid extract are in agreement. Gastric secretion in experimental cretinism has not been studied, but in Peacock and Dragstedt's (118) dogs, the thyroid as well as the parathyroids were removed without definitely influencing gastric secretion. The depression of gastric secretion by thyroid feeding is most probably due to its general toxic effects.

Pancreas: Extirpation of the pancreas in dogs causes hypersecretion of gastric juice (131). This is due chiefly to the exclusion of pancreatic juice from the intestine, because simple ligation of the pancreatic ducts has a similar, but not as marked an effect (132).

Ivy and Fischer (133) found that purified insulin does not excite gastric secretion and that it does not inhibit the gastric secretory response to a meal, a blood sugar concentration of 0.019 being compatible with the formation of normal juice. Collazo and Dobreff (134) made a similar observation so far as the response to a meal is concerned. Bustamante (135) studied gastric secretion in 29 patients and found that after insulin the free acidity was increased in 23, decreased in 3 and not changed in 3. Bowen and Aaron (136) made fractional analyses in sixty-nine diabetic patients. Achlorhydria was present in 20. A study of the duration of the diabetes suggested that achlorhydria may be the result of long standing or severe diabetes. McPherson (137) studied ten cases of severe diabetes mellitus; four showed achlorhydria and four others hypochlorhydria. In one case (in a young patient) after prolonged insulin treatment, the gastric analysis returned to a normal value.

Why this discrepancy between the effect of experimental and clinical diabetes? One explanation is that since extirpation diabetes is etiologically different from clinical diabetes, the etiological agent that is responsible for clinical diabetes is affecting the gastric glands. The effect of a long continued hyperglycemia on the gastric glands is not known; neither is the effect of ketosis known.

Adrenals: The effect of adrenalectomy on gastric secretion has not been adequately studied, but on the basis of the pathological changes occurring in the gastro-intestinal mucosa of such animals and the development of anorexia prior to death, one can predict a depression of gastric secretion (138) (139). Gastric secretion in Addison's disease is depressed or absent.

The literature on the effect of epinephrine on gastric secretion is reviewed in an article by Ivy and Javois (89) and by Sirontinin (140) and can be summarized by the statement that it has an uncertain effect, however administered, sometimes depressing, sometimes increasing, and sometimes having no effect on secretion. There is no evidence showing that epinephrine plays a physiological rôle in gastric secretion.

Pituitrine: Pituitrine in adequate amounts depresses gastric secretion (141). In acromegaly and Frolich's syndrome, gastric secretion is reported to be normal or decreased (124) (142) (143). It certainly plays no rôle in normal gastric secretion.

Gall bladder. It has been suggested that the gall bladder gives off a hormone that excites gastric secretion, because some workers have observed (144) (145) that removal of the gall-bladder diminishes gastric secretion in dogs. Meyer, Ivy and McEnery (146) were not able to confirm this, since cholecystectomy in their dogs either had no effect or slightly increased the secretory response to meals. They found a "gastrin-like" substance in gall-bladder mucosa, but expressed doubt of its physiological significance and concluded that changes in gastric secretion associated with gall-bladder disease are not due to the disturbance of some hypothetic hormone relation between the gall bladder and the gastric secretory mechanism.

Pancreatic secretion. Prior to the introduction of the secretin theory by Bayliss and Starling (49), physiologists believed that substances in the intestine excited pancreatic secretion through local reflexes from the intestine to the pancreas. This opinion was based on the observations of Bekker (147) and Dolinski (148) who found that the introduction of carbonic acid and mineral acids (HCl) into the intestine would stimulate the pancreas, and of Popielski (149) and Wertheimer

(150) who observed stimulation on introduction of acid into the intestine in animals, with the vagi and splanchnics cut, the spinal cord excised, and the abdominal sympathetic severed. Wertheimer and Le Page (151) added that the reflex diminished with descent down the intestine and that atropine did not abolish it. The latter group of workers showed that acid injected into the circulation was without effect. Bayliss and Starling, about this time, were interested in the local motor reflexes of the intestine and their interest was attracted by this local secretory reflex, which has not even today been demonstrated to exist. They started a research to study the conditions and paths of this local secretory reflex. First, they found that when acid is introduced into a loop of jejunum which had been denervated as far as possible, the pancreas would secrete. This suggested that the exciting agent was acting through the blood. They injected acid intravenously with negative results and concluded that the mucosal cells of the loop were giving off something to the blood. Second, they found that acid extracts of the duodenal and jejunal mucosa contained a substance called by them "secretin" which excited the pancreas to secrete on intravenous injection, the stimulation not being prevented by atropine. Because they were unable to extract secretin with cold water and alcohol, in which secretin extracted with acid is soluble, they concluded that the mucosal cells contain a "pro-secretin" which is converted by acid into secretin. The "secretin theory" was then proposed. This theory maintains that when the acid of the gastric chyme comes into contact with the upper intestinal mucosa, "pro-secretin" is changed into secretin which is absorbed into the blood and on reaching the pancreas causes it to secrete.

A number of investigators have repeated with variable results the experiment on the denervated loop. Wertheimer (152) and Luckhardt (153) obtained confirmatory results, but expressed doubt as to the completeness of the denervation. Popielski (154) obtained negative results when the pylorus was ligated. He believed that positive results when obtained are due to mechanical factors which have an effect on the movements of the contents of the stomach. Fleig (155), obtaining negative results, suggested that secretin might be formed but not absorbed from the denervated loop. Modrakovski (156) held that acid stimulation of the pancreas was due to a local nervous reflex. Babkin and Savitsch (157) found that atropine did not abolish the effect of acid in the duodenum.

Wertheimer and Le Page (151) and Fleig (155) found that venous

blood from a denervated jejunal loop containing acid would cause secretion of the pancreas when injected into another animal. They concluded, however, that both a peripheral reflex and a secretin mechanism were concerned. Enriquez and Hallion (158) reported one experiment in which they used carotid blood, obtaining a result similar to that of preceding investigators. Popielski (154) performed an experiment which seriously questioned the results of injecting blood from one animal into another. He found that the injection of blood from a "control" dog with a non-secreting pancreas may cause the pancreas to secrete. This was also found to be true for the stomach by Ivy, Lim, and McCarthy (75). A further attempt to demonstrate the presence of secretin in the blood was made by Matuso (159) who performed a carotid-to-jugular cross-circulation, obviously an unphysiological type of cross-circulation, and obtained secretion in both animals when he put acid into the duodenum of one. Luckhardt (160) using a syringe-cannula method, obtained negative results on cross-circulating a secreting with a non-secreting animal. This lack of uniformity and interpretation is not surprising when one considers the technical difficulties, the fact that the pancreas of some dogs under anesthesia is more refractory than others, and the results obtained by Ivy, Lim and McCarthy (75) in attempts to demonstrate a humoral mechanism for gastric secretion by blood transfusion and cross-circulation experiments performed under the best of conditions in unanesthetized animals.

The results of transplantation and vivi-dialysis have demonstrated unequivocally that after the ingestion of a meal, substances find their way into the blood which cause the pancreas to secrete. Hedon (161), Thirloix (162) and Laguesse (163) observed secretion in the auto-transplanted tail of the pancreas. They failed to observe any relationship between the ingestion of a meal and did not attach any significance to their observation. Ivy and Farrell (164) have observed that the auto-transplanted tail of the pancreas is stimulated to secrete on the ingestion of a meal, which proves a humoral mechanism. Ivy, Farrell and Lueth (165) transplanted two 6 inch loops of jejunum and the tail of the pancreas subcutaneously in the same dog and found that acid applied to the transplanted intestinal loops caused the transplanted pancreas to secrete. They interpreted this as proving that a hormone was liberated into the blood, since they applied a pure chemical substance (N/10—N/20 HCl) which parenterally does not stimulate the pancreas, to the intestine which had not been in contact with food for several weeks. Necheles and Lim (166) have by the method of vivi-dialysis shown that

a pancreatic secretory excitant is in the blood at all times which is increased in amount by a meal and is obtained more readily from the portal than from the carotid blood.

The evidence clearly indicates that there is not only a humoral but a hormonal mechanism for pancreatic secretion.

Specificity of secretin. The significance of the second observation of Bayliss and Starling, namely, that acid extracts of the duodenal and jejunal mucosa contain a specific substance which excites the pancreas to secrete, has also been questioned.

That this substance was not specific for the pancreas was maintained by Popielski (167) and others (168) (169) (97) because extracts of intestinal mucosa also stimulated the formation of gastric juice, urine, bile and saliva.

This position has been shown not to be true by a number of observations. A number of workers (170) (159), Mellanby and Hugget (71) and Skarzynska (172) have compared the action of drugs and tissue extracts of all kinds with the action of duodenal extract and found that the latter was much more potent as a pancreatic secretory excitant. Derouaux (173) found that if duodenal extract was treated with alcohol according to the method of Bayliss and Starling, it lost its salivary effect but not its effect on the pancreas. Others have found (174) (175) (170) that if various tissue extracts were treated with alcohol, they lose their general secretory effect, and cause only the pancreas to secrete. Further, Bayliss and Starling (49) said that they had observed secretion without a concomitant fall in blood pressure. Dale and Laidlaw (176) obtained extracts with much "secretin" activity and only slight blood pressure depressing action. Carlson, Lebensohn and Pearlman (177), Luckhardt and Blonder (178) and others showed that secretin was destroyed by peptic and tryptic digestion, the latter workers showing further that this treatment could destroy the secretin without changing the vaso-dilating properties of the extract. Parsons (179) showed by chemical analysis that histamine is not the vaso-dilator element of the Bayliss and Starling crude secretin. Further, Weaver, Luckhardt and Koch (180), Ivy, Kloster and Lueth (181), Mellanby (182) (202) (257), and Volborth (183) have prepared solutions of secretin free from vaso-dilating action. So it is an established fact that secretin action is not due to a general vaso-dilatin action.

Alleged secretin action has been found in extracts of many animal and plant tissues. In Witte's peptone (184), in stomach and colon mucosa (185) (186) (174), in muscle tissue (185) (186), in the brain

(187) (188), in the pancreas (187) (188), in the blood (187), in the thyroid (189) (190), in the liver (190) (191), in parathyroids, pineal and mammary gland (192), in spinach, nettle, cheese and in hydrolyzed egg white (62) (193) (194). Halliburton and de Souza (195) reported that spinach and hydrolyzed egg white caused only a weak response of the pancreas if the pylorus was obstructed. Dobreff (193) replied that they had not used the correct process, and Schimizu (196) reported that spinach extract causes a better secretion in pancreatic fistula dogs than in acute experiments.

All of this work on the alleged distribution of secretin probably means nothing from a physiological standpoint, since these extracts contain vaso-dilatin, and since we will not know whether their activity is due to secretin, or some other "pancreatic secretin," until we know the chemical identity of secretin. In this connection Drewyer and Ivy (197) have recently made extracts of various body tissues and spinach, using a method which uniformly yields secretin from duodenal mucosa and which rids the extract of vaso-dilatin, and have found that with the exception of the pyloric and colon mucosa they contain no pancreatic excitant.

Another observed fact which has engendered doubt as to the correctness of the physiological significance of a secretin and gastrin mechanism for pancreatic and gastric secretion, was that crude secretin, or crude gastrin, injected into the portal vein had little or no effect when compared to its effect on injection into the systemic veins (198) (195) (199) (200) (201). This was interpreted by Djenab (198) and Matsuoka (201) as showing that the liver protected the stomach and pancreas from over-excitation by the hormone or absorbed secretagogues and by others as questioning the hormone mechanism. However, these observations do not necessarily apply to the normal mechanism, since crude preparations were used. Mellanby (202) confirmed the above results for crude secretin, but showed that purified secretin would excite the pancreas as well on portal injection as on injection into the systemic veins.

Purified secretin does not stimulate the gastric glands according to Weaver (98). This has been confirmed by Burgess and Ivy (203) who have further found that purified secretin has no effect on the formation of succus entericus. Crude secretin augments the flow of bile (49) (204) (205). Lueth and Kloster (206) and Mellanby (207) found that purified secretin augments bile flow after a latent period of about ten minutes. Mellanby believes that secretin indirectly augments bile

flow through its action on the pancreas, because occlusion of the main pancreatic vein prevents the cholagogic action of secretin.

So secretin, after going through the vicissitudes of pharmacological and "physiological" investigation, according to the evidence at hand appears to be quite specific for pancreatic secretion.

Excitants of secretin production. The meager evidence at hand indicates that there is no secretin produced when various substances are applied to the stomach. Ivy and Lueth (208) using dogs with a pouch of the entire stomach and a duodeno-esophageal anastomosis and collecting the duodenal contents by tube, applied to the entire stomach such substances as dilute HCl, fat, meat extract, water, etc., without observing any augmentation of flow of secretion from the duodenal tube. Popielski (209) found also that introduction of acid in the fundic portion of the stomach had no effect on the pancreas. Tonkich (210), using animals with pancreatic fistula and various types of pouches and fistulae so that the substance to be tested could be introduced either into the pyloric antrum, fundus, or duodenum, arrived at the conclusion that fat and oleic acid caused as much secretion when placed into the pylorus so that it could pass into the intestine as occurred when these substances were introduced into the duodenum. Because the organic material in the juice was less when these substances were introduced into the duodenum, he concluded that the pylorus exercised a trophic action on the pancreatic secretory cells. Since it is well known that "secretin juice" is poorer in organic material than the secretion excited by the nerves, it is logical to assume that the pylorus does not produce much secretin, expecially since Drewyer and Ivy (197), using recently discovered methods for purifying secretin have found only small amounts of secretin in the pyloric mucosa.

Many substances introduced into the stomach so that they can pass into the intestine, or introduced directly into the intestine, excite pancreatic secretion. Gley (211) gives a summary of these substances known up to the year 1912. It includes many acids (dilute), neutral fat, mustard oil, chloroform, chloral, gastric juice, soap solution. To this list can be added water, peptone, albumoses, neutral salts, and meat extract (97), ether (213) (214), alcohol (97) (212) and bile (182) (229). Most of these substances are known to be present in the intestine during digestion.

The mechanism by which these substances stimulate pancreatic secretion is not known in every instance and it is only possible in this article for us to consider the most important of these: water, acid, fat,

soap, fatty acids, digested protein and bile. A number of possible mechanisms must be kept in mind. These mechanisms are as follows: *a.* These substances, on coming into contact with the upper intestinal mucosa, excite local reflexes which exert either a specific secretory influence on the pancreatic cells, or cause an increase in blood flow through the pancreas. *b.* These substances either contain, or on digestion yield, secretagogues that stimulate either by local reflexes, or after being absorbed into the blood, by action on the pancreatic cells or their blood supply. *c.* These substances, or secretagogues which might be present in them, on coming into contact with the upper intestinal mucosa, cause a hypothetical substance, "pro-secretin" present in the cells of the mucosa, to be converted into secretin, which passes into the blood and is carried to the pancreas and stimulates it, either by direct action on the cells or by causing an increased blood flow and capillary permeability, this being the mechanism that is commonly known as the Bayliss and Starling secretin theory. *d.* These substances may affect the motility of the upper intestine which in turn, by reflexes or by an increase in blood flow, may excite the pancreas. *e.* Some substances may act by tending to cause hydremic plethora. Also a change in motility may play an indirect rôle by promoting the absorption of secretagogues, or the passage of a hormone stored in the mucosal cells, into the blood. Any one, or all of these possible mechanisms, must be considered in analyzing how any single substance excites the pancreas to external secretion when introduced into the intestine.

Water. Water stimulates pancreatic secretion directly via the intestine and indirectly through its excitation of the gastric glands, which has been demonstrated by the observations of Damskin (215), Babkin (216) and Bylina (217) and Cohnheim and Klee (218).

The direct action of water on the pancreas is observed after a latent period of from three to five minutes and the maximum effect (300 to 500 cc. of water) is reached at about one hour. The observations of Ivy, Farrell and Lueth (165) indicate that water does not act by exciting the formation of secretin, for when they perfused a Thiry fistula of the jejunum in a dog with a pancreatic transplant, the transplant was not stimulated. Water probably acts either by increasing the action of bile, by facilitating the absorption of secregatogues, or by tending to produce an hydremic plethora.

Digested proteins. Bylina (217) has reported that, if the activity of the gastric glands is avoided by the production of an experimental achylia, 300 cc. of a 5 per cent meat extract has no more effect on the

pancreas than so much water; and meat eaten with appetite caused more pancreatic secretion than when given by stomach tube. A 1 per cent peptone solution had about the same effect on the pancreas as water (219) (220). The various amino acids that have been studied do not stimulate pancreatic secretion when given subcutaneously or intravenously, and when given by mouth or intestine have a questionable stimulating action, which is very likely due to the acid in the acid salts of the amino-acids used (221) (222) (223). The action of various amines has not been studied extensively. Histamine and d-glucosamine hydrochloride excite pancreatic secretion on application to the intestine (221) (222); and tri-methyl amine, tetramethylammonium chloride, histamine and choline excite intravenously (224) (225). But it is a question whether any of these amines plays a physiological rôle because we do not know their concentration in the intestinal contents and the facts governing their absorption and mode of action.

It appears from this evidence that the proteins excite pancreatic secretion primarily if not entirely through their effect on the gastric glands, or the excitation of the pancreas by proteins is due to the gastric secretion they excite acting in the intestine. We have no data on the effect of meat extract and peptone applied to a Thiry fistula on a pancreatic transplant.

Starch. According to all investigators the introduction of starches, glucose and bread into the intestine does not stimulate the pancreas unless the animal voluntarily and with appetite eats these foods. Bylina (217), Krewer (226) and Walther (227) in Pavlov's laboratory performed some experiments showing that bread increases the amolytic activity of the secretion, but this has not been confirmed or proven by a sufficient number of experiments. Ivy and Farrell (164) found that starch (with water) by mouth caused only a slight stimulation of secretion in the transplanted pancreas. Cooked starch and glucose applied to a Thiry fistula does not cause secretion in the pancreatic transplant. The maximum pancreatic response to a carbohydrate meal is reached at the end of one hour. Therefore it appears as if carbohydrates excite pancreatic secretion only through the appetite secretion of gastric or pancreatic juice they call forth.

Fat, soaps and fatty acids. Dolinski and Damaskin (148) (215) found that olive oil would stimulate the pancreas and that the latent period was from three to five minutes. Babkin and Ishikawa (228) introduced olive oil into the non-secreting stomach through a fistula and observed it leaving the stomach (making observations through a

duodenal fistula) two minutes later. Six minutes after introducing it into the stomach and four minutes after it entered the intestine, augmentation of pancreatic flow was observed. Bylina (217) showed that this "quick" action of fat was not due to acid being passed from the stomach into the intestine. Ivy, Lueth and Oldberg (229) observed stimulation within ten minutes on introducing fat into the stomach by tube, but not as uniformly as they had been led to believe by reading the literature. They also observed stimulation following the same procedure in dogs with a pancreatic transplant within fifteen minutes on some occasions but did not control the gastric juice factor. This observation does show, however, that fat operates in some manner to give rise to a humoral agency. If a Thiry fistula of the jejunum is perfused with olive oil for one-half to one hour, in a dog with a pancreatic transplant, no evidence of stimulation is observed (229). Popielski (232) and Studzinski (230) held that the "quick" response of the pancreas to fat was due to fatty acids in the fat or produced rapidly by digestion, which was shown not to be the case by Bylina (231) and by Babkin and Ishikawa (233) (239) (240). How rapidly digestion goes on in the intestine is not known but it is not very likely that fatty acids or soap can be produced and cause stimulation within five minutes.

The failure of fat to excite the pancreatic transplant when the fat is applied to a Thiry fistula strongly indicates that fat *per se* does not cause secretin to be formed. An examination of the ferments and organic material of the juice secreted on fat stimulation according to the studies of Bylina (234) indicates that fat excites in part by a nervous mechanism, since "fat juice" is richer than "soap juice," and the injection of atropine does not change the quantity of "fat juice" but does change the quality. Babkin (96) has concluded that nerves play an important part in fat stimulation of the pancreas. Fleig (235) expressed a similar view because he could not show secretin formation *in vitro* by extracting mucosa with neutral fat, nor *in vivo*, and emulsified fat intravenously failed to stimulate. A factor that has been overlooked by the early investigators is that fat may cause the "quick," or preliminary, excitation of the pancreas by causing a flow of bile, which excites a formation of secretin. According to Fleig (236), bile begins to flow into the intestine within five minutes after the introduction of fat, and it is known that bile stimulates pancreatic secretion. Ivy, Lueth and Oldberg (229) found in six experiments on pancreatic fistula dogs with the bile duct ligated that the "quick" response of the pancreas to olive oil did not occur. They hesitated to draw a definite conclusion from

their observations because of the irregularity of the "quick" response of the pancreas to fat and bile, a statistical study being necessary to establish such a point.

The delayed, or secondary stimulation by fat has been shown (96) to be due in part to a secretin-like mechanism set up by fatty acids and soap acting in the intestine. Glycerine does not stimulate the pancreas (216) (237). Fleig (238) and Camus and Gley (224) were able to extract secretin from intestinal mucosa with soap solution. Atropine decreased the response to such extracts, as well as the response to soap in the intestine (224) (238) (239) (240) (234) (233). Babkin (96) has suggested that soap on being absorbed into the blood takes along substances which act on both gland cells and nerve endings, atropine causing a decrease by paralyzing the nerve endings. That a humoral mechanism is concerned in the action of fatty acids, soaps or digested fat is not shown by the stimulation of a pancreatic transplant when digested ("pancreatin") olive oil is applied to a Thiry fistula of the duodenum (229) because soaps and fatty acids applied to a Thiry fistula excite gastric secretion (95) and bile flow, which was not controlled in the above experiments and has not been avoided in much of the work on soap stimulation. The fact remains, however, that digested fat in some manner gives rise to a humoral agency for pancreatic secretion. Much work is yet to be done before the mechanism of fat stimulation of the pancreas is thoroughly understood.

Acid stimulation. Giving dilute hydrochloric acid by mouth to an animal with a pancreatic transplant causes the transplant to secrete. The application of acid to a Thiry fistula of the jejunum causes the transplant to secrete, and the application of acid to a transplanted loop of jejunum causes the pancreatic transplant to secrete (164) (165). The latter observations prove that when acid comes into contact with the upper intestinal mucosa something gets into the blood which excites the pancreas. This has been further substantiated by Necheles and Lim (166) who were able to obtain a pancreatic excitant from the blood vivi-dialysate after the administration of food or acid. The conclusion can hardly be avoided that acid stimulates the pancreas by a hormone mechanism.

Bile stimulation. Mellanby (182) (241) has shown that the introduction of dilute bile, of an adequate reaction into the duodenum of the cat stimulates the pancreas (latent period 2–54 minutes) even when the pyloric sphincter is tied, which must be done because it has been shown by Meyer, Ivy and McEnery (146) that bile in the intestine stimulates gas-

tric secretion. Mellanby's observations were confirmed by Oldberg and Ivy (242) in cats.

It is obviously significant that the bile must be slightly alkaline (pH 7.8) in order to excite the resting pancreas but after the pancreas has been excited bile of various reactions may continue to stimulate the pancreas (optimum, pH 6.5). According to Mellanby the cholic acid is the active constituent of bile, but its activity is modified favorably by its association with taurine and glycine. Lueth and Ivy (208) found that bile when applied to a Thiry fistula of the jejunum caused a pancreatic transplant to secrete, but not invariably. If the flow of juice is rapid to start with, bile so administered sometimes caused a decrease. This variation was independent of the reaction of the bile in animals which had not been fed for 18 hours. Bile by stomach tube did not stimulate as uniformly as N/10 HCl. Mellanby found that bile would stimulate after ergotamine and atropine and concluded that the bile was not acting via some nervous mechanism. But there may be some local nervous mechanism in the intestine not affected by these drugs. The observations on the transplanted pancreas, however, demonstrate that bile operates by some humoral mechanism. Mellanby did not inject bile salts to find their effect on pancreatic secretion. Drewyer (243) has found, however, that they do not stimulate the pancreas of the dog. Mellanby suggested that bile is "the intestinal stimulus for pancreatic secretion" and that the bile salts on passing through the intestinal mucosa into the blood adsorb the preformed secretin. Ivy, Lueth and Oldberg (229) compared the pancreatic secretory response of dogs with a pancreatic fistula and a pancreatic transplant to a meal of meat (half lean and half fat) before and after ligating the common bile duct. They found that the exclusion of bile had no appreciable influence on the response to such a meal. One fistula animal with the common duct doubly ligated and cut, on eating a meal, secreted at the rate of 40 cc. of pancreatic juice per hour for five hours. They concluded that bile was only an adjuvant and not an essential alimentary stimulating agent, the pancreas being adequately stimulated by food in the absence of bile.

Secretagogues as the humoral agent. It has been pointed out in the discussion of how proteins excite the pancreas that it is not likely on the basis of the evidence at hand that the protein derivatives stimulate the pancreas under the normal conditions of digestion. Emulsified fat intravenously has no effect on the pancreas. Other than secretin, only drugs or substances that cause quite a fall in blood pressure are known to excite the pancreas to secrete.

The correct answer to this question can only be attained after the chemical identity of secretin is known and methods for its quantitative assay have been discovered.

How is secretin formed? Bayliss and Starling (49) because they were unable to obtain an active preparation of secretin by extracting the intestinal mucosa with cold water, alcohol, or salt solution concluded that the cells lining the gut contain a substance,—"pro-secretin,"—which under the influence of such agents as acids undergoes hydrolysis with the production of secretin. They believed that this chemical mechanism is normally involved because the amount of secretin obtained in extracts decreases as one proceeds down the intestine. This latter statement has been confirmed a number of times, most recently by Mellanby and Huggett (171). The first statement has been shown to be incorrect since secretin can be extracted with sodium oleate (238), chloral hydrate (244), sodium chloride (245), cane sugar, glycine, urea and soaps (246), ethyl alcohol (247) (171) (248), dilute NaOH, acid and alcoholic phosphate, dilute NaCl water, and acetone solutions (171) (241). The most recent preparations have been "dilatin" free. Thus there are numerous solvents that can extract secretin from the duodenal mucosa. But this does not prove that pro-secretin does or does not exist.

In an article in 1913, Starling (249) did not insist on the existence of pro-secretin and expressed the opinion that secretin may be present in the cell bound in some way to the protoplasm, the union being broken up by different physiological excitants, or by various methods of extraction after the death of the cell.

Food is not necessary to cause the formation of secretin by the intestinal mucosa, since it is found in the duodenal mucosa of the fetus (250).

The mechanism of secretin production is difficult to exhaust. Grogan and Luckhardt (251) induced pancreatic secretion repeatedly by introduction of 0.2 per cent HCl into the duodenum. The secretory response of the pancreas gradually decreased and almost ceased, but the injection of secretin intravenously caused an excellent response. The extract of the mucosa so treated yielded a normal amount of secretin. Ivy and Javois (252) performed the same type of experiments with the same results. Grogan and Luckhardt found further, however, that if 0.4 per cent acid was used instead of the weaker acid, practically all the secretin went into the acid washings. Mellanby (182), using bile as a pancreatic excitant, also found that the secretin mechanism was difficult to exhaust.

Atropine does not prevent the stimulation of the pancreas caused by the introduction of acid into the duodenum (253) (254) (157). Ivy, Farrell and Lueth (165), however, observed that atropine (1.5 mgm.) decreased the response of the pancreatic transplant when acid was introduced into a Thiry fistula, but not when given by stomach tube, the explanation of which is not clear. Atropine decreases the response of the pancreas to a meal (255) (256), which is accounted for by the decrease in gastric secretion. Mellanby (257) found that neither atropine nor ergotamine prevented bile stimulation of the pancreas. Thus, there is no evidence, as there is in the case of gastrin production, suggesting that secretin production is under the control of nerves.

How does secretin act? Secretin preparations cannot be administered by mouth, since it is well known that pepsin and trypsin (177) (178) rapidly destroy it. Mellanby (257) has reported that occasionally it will stimulate very mildly on subcutaneous injection. All observers agree that to cause an active secretion, secretin must be given intravenously.

The evidence indicates that secretin does not act on parasympathetic secretory nerve endings. It is well known that atropine does not abolish secretin action and as a matter of fact may sometimes increase its action. Hence one might conclude that secretin does not act on vagus nerve endings. But since the vagus apparently contains both "inhibitory" and secretory fibers (47) (96), atropine may affect the two differently. This must be considered because atropine not infrequently augments temporarily the continuous or basal flow of juice from the pancreas, this being especially true for large doses (256) (254). The work of Anrep (258) has a bearing on this question. He found that at first the stimulation of the peripheral vagus causes no secretion, but on repeated stimulation, or probably on fatigue of the "inhibitory" fibers, secretion occurs. (The vagus according to Anrep contains no vasomotor fibers for the pancreas.) If the gland is secreting, stimulation of the vagus causes the gland to dilate due either to contraction of the ducts, or to retention of juice in the glands, or to activity of the "inhibitory" fibers. Atropine (2.0 mgm. doses) does not paralyse the "inhibitory" fibers, but does the secretory fibers. So, if atropine stops secretion, stimulation of the vagus now does not cause the gland to dilate. But if the secretion is evoked by secretin, which is not affected by atropine, stimulation of the vagus decreases the rate of secretion and the gland dilates. This indicates that secretin does not act on either the vagus secretory or "inhibitory" fibers.

Edmunds (259) found that secretin action is unaffected by both adrenalin and ergotoxin, if the adrenalin is given and then followed by ergotoxin, or given in such a manner as not to affect blood pressure or lead to vaso-constriction of the pancreas. Mellanby (182) has shown that bile stimulation occurs after ergotoxin, but whether the ergotoxin changed the rate is not decisively shown by his data. From the meager evidence at hand, we cannot state whether secretin affects the sympathetic nerve endings of the pancreas or not, especially since in all the work done on this subject crude secretin was used which contains "vasodilatin" and complicates the results. This statement is more true for the work on the sympathetic than for the work on the vagus, because the latter does not contain vasomotor nerves for the pancreas (258).

If the secretion of the pancreas is uniformly excited by a *continuous* injection of secretin, all drugs, such as epinephrine (1–500,000) ergotoxin, nicotine, pituitrine, strophanthin, strychnine and barium chloride (259) (260) (261) (262) (263) which cause a rise in blood pressure, vaso-constriction of the pancreas, or procedures which cause a decrease in the blood supply of the pancreas, inhibit pancreatic secretion. Mann and McLachlin (260) even observed inhibition of pancreatic secretion caused by secretin on the injection of a dose of epinephrine which causes a fall in general blood pressure, which however caused a decrease in the volume of the gland, showing that the blood vessels of the pancreas are especially susceptible to the vaso-constrictor action of epinephrine. The pancreas, then, is epecially susceptible to changes in blood flow, which has been demonstrated very convincingly by Babkin (264), using various physiological procedures instead of drugs, even when the secretion is excited by secretin. Barlow (265) has verified the importance of the relation of blood flow to the secretory response of the pancreas to secretin by showing that vaso-dilatins act synergistically with secretin.

If secretin does not act on nerve endings, it may act by augmenting blood flow or by acting directly on the cell, or both. The older evidence on this question is valueless because the secretin used contained much "dilatin." More recent evidence is contradictory. Burton-Opitz (266) found an increase in splanchnic organ volume as well as an increase in venous pressure and blood flow in the gastro-duodenal artery on introduction of acid into the duodenum without an appreciable change in general blood pressure. It does not necessarily follow, however, that this increase was due to secretin. Barlow (265), using a preparation of purified "new secretin" free of dilatin, i.e. having either no effect on

blood pressure or raising it slightly in the doses used, found an increase in kidney volume. He performed only a few preliminary experiments on the pancreatic volume, and finding that the change in the pancreas was similar to that of the kidney, he used the kidney volume change as an index of pancreas volume change. Mellanby (257) concluded that purified secretin has no action on the arterial blood pressure, although it produces local vaso-dilatation in the pancreas. He stated, however, that a large dose of his secretin caused a transient fall (does not give amount) of blood pressure. For change in vascularity of the pancreas, he used the method of direct inspection. The observations of Barlow and Mellanby are not conclusive, especially in view of the observations of Weaver (267), who used vaso-dilatin free (in the dose used) secretin. Using the plethysmographic method, he found only a small increase in kidney volume and no change in splenic and intestinal volume. He also cannulated the pancreatic tail vein in an heparinized dog and observed no increase in blood flow on the injection of a stimulating dose of secretin.

It is impossible to state at the present time whether or not secretin acts by increasing blood flow to the pancreas. From Weaver's results with the direct method of counting blood drops, it appears as if secretin does not act primarily by increasing blood flow. The old results in the literature on the effect of secretin on the composition of juice cannot be used for evidence because secretin containing much "dilatin" was used. And if vaso-dilatin free secretin should produce the same results, we would be no closer to the solution of the problem than we are to the solution of the problem of how pilocarpine acts on the salivary glands to produce a thin saliva.

How is secretin prepared? A definite step forward in the preparation of secretin was made by Weaver, Luckhardt and Koch (180) when they discovered secretin activity could be separated from the vaso-dilatin activity of duodenal extracts by sodium chloride precipitation, which is a very reliable procedure when their directions are properly followed. A method for the purification of secretin was discovered by Mellanby (257) in which the secretin is extracted with absolute alcohol and precipitated with bile salts, a method that yielded a very pure secretin active in the cat in doses of 0.05 to 0.017 mgm. of dried material. Mortimer and Ivy (248) on using this method found that it was unreliable, due chiefly to the lack of uniformity of the "bile salt" precipitation procedure. The method possesses considerable promise because when it works it yields a highly purified product practically "dilatin" free and active in doses of 0.3 mgm. in a 30 to 35 pound dog.

Evaluation of the hormone mechanism for pancreatic secretion. Since so little juice is produced during the cephalic phase of pancreatic secretion (208), and since there is no good evidence showing a local nervous mechanism in the stomach or intestine for pancreatic secretion, and since the pancreatic transplant yields an active secretion, the statement is warranted that the humoral mechanism plays the most important rôle in the excitation of the pancreas to secrete its digestive juice. Since there are a number of substances present in the intestine during digestion which are capable of calling forth the humoral mechanism, the facts that the acidity of the upper intestine is not very great, and that a patient with achylia gastria, or a dog without a stomach is able to digest food, can no longer be used as argument against the existence of a humoral mechanism.

Whether the humoral agency is hormonic or secretagogic awaits further investigation of a chemical nature, but the direct physiological evidence for the existence of a hormone mechanism is almost incontrovertible.

Effect of endocrine glands on pancreatic secretion. Adrenals: Sweet and Pemberton (268) observed that following the removal of the adrenals a marked augmentation of pancreatic secretion occurred. They attributed this to some specific relation that the adrenals had to pancreatic secretion, and reported that epinephrine (not the pure substance) contained a specific inhibitory substance for the pancreas other than its vaso-constrictor substance, which was removed from the body by the adrenal extirpation. Edmunds (259) could not confirm this latter statement and suggested that the increase in pancreatic secretion was due to removal of vaso-constrictor tone due to shock and low blood pressure and not due to some specific relation of the adrenal to the pancreas. Sweet and Pemberton found that the removal of one adrenal had little or no effect, whereas the removal of both caused the "marked flow." These animals were apparently very much traumatized because they state that "animals that have been subjected to bilateral ablation of the adrenals withstand poorly any further trauma, even that of establishing a connection with the respiratory tambour or blood pressure manometer," which demonstrates that their dogs were very abnormal.

What might be the effect of the normal maximal output of epinephrine has not been determined. Mann and McLachlin (260) observed that 10 cc. of 1–500,000 epinephrine would decrease the pancreatic response to secretin. If the output under emotional conditions (269) should

approximate this amount, it is reasonable to believe that pancreatic secretion would be decreased. This is mentioned because Ivy and Farrell (164) observed a decrease in the response of the pancreatic transplant when their dogs were distressed, which is also true of the pancreatic fistula dog, the latter being explained as due to either splanchnic constriction or action of inhibitory fibers in the vagus. Ivy and Farrell suggested that in the pancreatic transplant it might be due to constriction of the vessels of the mammary gland which supply the transplant with blood. Another explanation might be that the acid (given by tube) was retained longer in the stomach due to pylorospasm resulting from the distress.

The literature records no tests of pancreatic function in Addison's disease.

Thyroid and parathyroids: Pemberton and Sweet (270) found that animals in which the thyroid-parathyroid apparatus had been removed with the ensuing tetany, responded in a normal manner to secretin. Stoland (271) has shown that in parathyroid tetany the secretion of pancreatic juice and bile to a meal is diminished; but from the results of Peacock and Dragstedt (118) on the presence of normal gastric secretion in animals in which the tetany is controlled it is logical to predict that pancreatic secretion would also be normal under such conditions.

Pancreatic function in clinical or experimental "hyperthyroidism" has not been studied.

Insulin: The clinical literature is agreed that the external secretory activity of the pancreas is definitely decreased in grave cases of diabetes (272) (273) (274). Jones, Castle and Mulholland (272) report that effective insulin therapy appeared to reduce the external pancreatic secretory disturbance, a marked improvement occurring on relief of the acidosis. The effect of ketosis on pancreatic secretion has not been studied experimentally. The clinical action of insulin is in all probability not due to any specific action of insulin on the external secretory mechanism of the pancreas, but is due to the general action of insulin in improving or correcting the disturbed nutrition of the cells incident to the diabetic condition. However, Collazo and Dobreff (275) report an increase in pancreatic secretion on the intravenous injection of insulin. The stimulation was probably due to substances other than insulin in the preparation they used, since Lambert and Hermann (276) failed to observe augmentation of secretion in pancreatic fistula dogs under the influence of insulin.

BILE FORMATION. That a humoral mechanism is concerned in the formation of bile is unquestionably established. Bile salts are absorbed from the lumen of the intestine and act as the most potent of cholagogues (277). This is the most definite example of the secretagogue type of glandular stimulation we possess.

That the character of food ingested has an important influence on the volume of the bile formed was first clearly established by contributions from Pavlov's laboratory (47), meat having a definite augmentive action, fat less, and starch or sugar having either no stimulating or an inhibitory action.

How foods exercise their action was a question that puzzled Pavlov, and is still a puzzle today. As is the case in the formation of the other digestive secretions the following factors must be considered: 1, increased blood flow *per se;* 2, secretagogues in the food or produced by digestion; 3, a hormone, by serving as a direct cholagogue or by augmenting blood flow; and 4, a reflex nervous mechanism, affecting the liver cells directly or by increasing blood flow. In studying these factors the absorption of bile salts must be avoided.

The discovery of Rutherford in 1880 (278) that the introduction of acid into the duodenum provokes a flow of bile from the cannulated common bile duct attracted no special interest until the discovery of secretin by Bayliss and Starling, when the observation was abundantly verified (236) (244) (279) (280) (281) (282), both by "acute" and "chronic" experiments. And as is the case in pancreatic stimulation, the lower down the gut the acid is introduced, the less the effect on bile formation. Bayliss and Starling (49) found that the injection of crude secretin intravenously after a latent period of five minutes or more increases the formation of bile. They used a preparation of secretin which contained only a small amount of depressor substance. This was confirmed by a number of observers (283) (284) (204) (281) (285) (286) (287). Okada (281) used a gall-bladder fistula animal with the common bile duct tied and observed that secretin almost doubled the formation of bile. The effect of the injection lasted about an hour; and if the secretin activity had disappeared from the solution, the solution had no effect on bile formation. Fleig (214) (288) found that blood from an isolated loop of duodeno-jejunum into which he had put acid on injection into a second dog increased the flow of bile. In all the above experiments in which secretin was used to excite the flow of bile there was present some vaso-dilatin material and possibly some bile salt at least in some of the preparations. This causes one to be skeptical

concerning the meaning of such results in spite of Okada's observations. Purified secretin free of "dilatin" and bile salts has been injected intravenously to ascertain its effect on bile flow by Mellanby (207) and Lueth and Kloster (206). The latter workers found that the injection of their purified secretin (1.0 mgm. in a 30 lb. dog, cystic duct tied and common duct cannulated) caused a copious pancreatic secretion within one to three minutes, and a definite augmentation of bile flow after a latent period of seven to nine minutes, the augmentation in bile flow never amounting to more than ten per cent of the augmentation in pancreatic flow. Mellanby obtained similar results and had his attention attracted by the long latent period which all have observed. His experimental analysis of the mechanism concerned in "secretin stimulation" of bile formation, in which he removed the intestine and clamped the pancreatic veins, led him to the conclusion that the action of secretin as a cholagogue is secondary to its action on the pancreas, the metabolic products of the pancreas being the biliary excitants. This finding will explain the observation on the effect of acid in the intestine on bile formation, but will not explain Fleig's (288) result on obtaining a biliary excitant from the blood of an intestinal loop with acid in it. It is possible, however, that the acid in the loop causes the production of metabolic products by the intestinal cells analogous to those in the pancreas. From Mellanby's work, it appears that we can no longer speak of secretin as a direct cholagogue, but this question is not to be considered as settled.

If secretin is not responsible for directly augmenting bile secretion, how do certain foods exercise their effect? It is true that numerous substances such as soaps, salts, glycerine, meat extract, digested egg white, albumoses, peptones, etc., introduced into the intestine increase the production of bile. But as is the case of secretin, their effects are small when compared to bile salts and may be explained on the basis of Mellanby's explanation or on changes in blood flow through the liver. It is striking, as pointed out by Pavlov, that those substances that are potent excitants for the pancreas also have an augmentatory action on bile formation. There is no evidence free from serious objection that supports the existence of cholagogues of the secretagogue type in such substances, and the possibility of specific nervous influence is far from having been proved. These substances lead to gall-bladder evacuation and it might be suspected that the emptying of the bile from the gall bladder furnishes an extra supply of bile salts which cause the increased volume of bile formation. This, however, is not true because the effect

of such substances is manifested by animals with a gall-bladder fistula and the common duct tied. So, in order to explain the effect of stimulating foods we must assume either that the bile forming hormone has not yet been discovered, or that these foods affect bile formation indirectly by their total or integrated action on the activity of the stomach, pancreas and intestine. The latter possibility is sufficient to explain the difference in action of meat, fats, and sugar and starch, meat causing activity both of the gastric and pancreatic glands, fat causing primary depression of the gastric glands, but much pancreatic activity, and sugar and starch causing very little gastric and pancreatic activity.

The effect of various drugs, of endocrine products (adrenalin, pituitrine), of endocrine extirpations, etc., on bile formation are best explained, in the light of the evidence at hand, as due to changes in blood flow through the liver or to changes in the activity of the upper portion of the gastro-intestinal tract (277).

INTESTINAL GLANDS. Most observers are agreed that intestinal secretion is best evoked by local mechanical and chemical stimulation and that increased motility and secretion are closely related, the most normal method for obtaining a good flow of intestinal juice from a Thiry fistula being to insert a rubber tube into the fistula. This speaks more in favor of a local nervous than for a chemical mechanism.

However, considerable evidence has been advanced in support of a humoral or a hormonal mechanism. The ingestion of a meal causes a slight secretion beginning about three hours after the meal and lasting about seven hours (204) (96). Delezene and Frouin (204) found that the introduction of dilute acid into an isolated loop caused secretion in a second loop of duodeno-jejunum. They also found that acid, soap, ether and chloral extracts of intestinal mucosa on intravenous injection caused a secretion in a Thiry fistula of duodenum or jejunum and obtained a greater response in the fed than in the fasting dog. Botazzi and Gabrieli (203), Mironescu (289), Hirata (290), and Komaroff (291) have injected intravenously various tissue extracts, plant extracts, meat extracts, observing stimulation in most instances. These extracts lower blood pressure and excite motility of the intestinal loop. Pilocarpine and especially histamine (292) (293) have a similar action and increase the secretion of the loop. So, the common factor concerned in these observations appears to be splanchnic hyperemia and augmented motility.

Frouin (294) found that the intravenous injection of intestinal juice itself caused a Thiry fistula to secrete, which may be explained by the

fact that secretin has been found in the contents of a Thiry fistula, or by the reduction of blood pressure the injection of intestinal juice causes (183) (251) (252). However, there is no evidence showing that secretin is absorbed from the lumen of the intestine. The intestinal transplants of Ivy, Farrell and Lueth (165) were motile and secreted to mechanical and chemical stimuli applied locally, but no definite increase in activity was noted after ingestion of food. It must be kept in mind that the blood supply of such transplants is decreased and they may not respond to minimal stimuli. Burgess and Ivy (203) find that the effect of purified secretion on the secretion and motility of a Thiry fistula of the jejunum is variable. Sometimes an increase, sometimes a decrease, and sometimes no change is observed, the interpretation of which is not clear. The fact that a denervated loop of intestine secretes more copiously than an intact loop (295) (296) (297) has been interpreted by Komaroff (291) as showing that when the inhibitory influence of nerves has been removed, various secretagogues in the blood excite the intestinal glands. This observation the author believes is still best explained by the hyperemia that occurs in such a denervated loop (298), since the more recent work of Savitsch (297) to the contrary is not entirely convincing. However, an open mind on the subject should be maintained, since the negative evidence is not final.

GALL BLADDER. In the latter part of the 18th century (1787), the professor of anatomy (307) in the University of Edinburgh taught that the gall-bladder receives the bile when the stomach is empty and evacuates it during digestion. Michael Foster (299) in his text in 1895 taught that the acid contents of the stomach when ejected into the duodenum cause a contraction of the gall bladder and its ducts with an expulsion of bile. Okada (300), working in Starling's laboratory, found that the ingestion of a meal and the introduction of dilute acid caused an increase in the tonus rhythm of the gall bladder, which has been confirmed (301).

Braga and Campos (302) reported that an injection of a secretin solution caused gall-bladder evacuation. Windlestein and Aschner (303), Whitaker (304) and Copher and Illingworth (305) obtained negative results with secretin solutions. Ishiyama (306) has reported that secretin causes a flow of gall-bladder bile, and stated that he was able to obtain a "hormone" from gall-bladder extracts and chyle which he termed choline-like in nature. Boyden (307) found that the transfusion of blood of fed or starved cats caused a partial evacuation of the gall-bladder. He interpreted the results of his experiments as meaning

that the gall-bladder is sensitive to bodily conditions which affect changes in the circulating blood. Whitaker (304) (308) found after a serious attempt to denervate the gall bladder that egg yolk and cream would lead to gall-bladder evacuation. Brugsch and Horsters (309) observed a contraction and rhythmic changes of the gall-bladder after the injection of "secretin." The former observed that histamine caused the gall-bladder to contract and concluded that histamine and "secretin" act alike, the latter being the physiological agent. They did not make a simultaneous blood pressure tracing to rule out vaso-dilatation and liver volume change as factors in causing gall bladder pressure changes. Mellanby (241) reported that strips of gall bladder suspended in oxygenated Ringer's solution could be caused to contract by adding a trace of "pure" secretin. Ivy and Oldberg (310) found that a purified secretin preparation free of vaso-dilatin when injected intravenously would cause the gall bladder to contract and to evacuate. Their preparations had no objective toxic action on unanesthetized dogs. By cross-circulation experiments they showed that the injection of acid into the duodenum of one dog not only caused his gall bladder to contract, but also caused the gall bladder of the other dog to contract. They showed that the active principle was not histamine or cholin and proposed the term "cholecystokinin" to designate the active principle in extracts of the intestinal mucosa which causes the gall bladder to contract. Lueth, Ivy and Kloster (311) observed the gall bladder contract and evacuate under direct vision and demonstrated that the changes in the gall bladder were not due to changes in liver volume. They further observed that the active principle in the doses used affected neither the motility of the stomach nor intestine in lightly anesthetized dogs. Burgess (312) found that in the unanesthetized dog it had no effect on gastric motility and a variable effect on intestinal motility. Burget (313) has found that a cholecystokinin solution will not only cause the gall bladder of the guinea pig to contract but also the duodenum Kendall (314) found that it would also cause the isolated intestine of the guinea pig to contract. Lueth, Ivy and Kloster (311) were unable to show that the gall bladder of the rabbit would contract on the injection of a cholecystokinin preparation. Ivy, Drewyer and Orndoff (315) have shown recently that the gall bladder of man responds to cholecystokinin. Atropine (3 mgm.) and ergotamine (1.5 mgm.) did not materially influence the response of the gall bladder to cholecystokinin in the cat and dog.

The author believes that it is difficult to interpret the evidence at hand

in any other way than as showing that a hormone mechanism is concerned in gall-bladder contraction and evacuation. This is true for the dog, cat and man. But it remains to be determined whether or not it is true of the rabbit.

Source of cholecystokinin. Ivy, Kloster, Lueth and Drewyer (316) have found "cholecystokinin" to be present in the upper intestine of the hog, dog, sheep and cattle. A small amount could be extracted from the stomach, some attempts being attended with negative results. They extracted liver, kidney, spleen and egg yolk without finding the active principle. Drewyer (317) has also found it in the upper intestinal mucosa of man.

Is cholecystokinin different from secretin? Ivy, Kloster, Lueth and Drewyer (316) believe that "cholecystokinin" is different from secretin because they were able to prepare secretin solutions free, according to biological assay, from "cholecystokinin" and solutions of "cholecystokinin" free from secretin. Further knowledge of the chemistry of these two active principles is required before it can be stated with certainty that they are different.

The chemistry of cholecystokinin will not be reviewed because so little is known concerning it and all that is known is given in the article by Ivy, Kloster, Lueth and Drewyer (316).

Evaluation of the hormone mechanism for gall bladder contraction and evacuation. It is impossible with the evidence at hand to evaluate the rôle that the hormone mechanism plays in gall bladder evacuation. But it is quite certain that this mechanism is not the only one concerned. The rôle played by the "sphincter of the gall bladder" (318) (319), by nerves, and by the tone of the sphincter of Oddi (320) (305) (321) (301) (322) must be considered. This is shown by the fact that the gall bladder can only exert a pressure of 20 to 30 cm. of bile pressure, whereas the pressure required to force bile from the common duct into the duodenum may vary from 5 to 70 cm. of bile pressure. The effect of cholecystokinin on the sphincter of Oddi or the "sphincter of the gall bladder" has not been determined.

It is a propos to point out, but not discuss, in this connection that Sweet (323) and Halpert and Hanke (324) doubt that the gall bladder normally evacuates, which may be true for the rabbit, which was used by Halpert and Hanke. The evidence abundantly supports, if it does not prove, the view that the normal gall bladder of the cat, dog and man does evacuate at least partially under appropriate stimulation.

MOTILITY OF THE GASTRO-INTESTINAL TRACT. A number of inves-

tigators have suggested that the motility of the gastro-intestinal tract is controlled by a hormone mechanism. Heidenhain (325) observed that the intravenous injection of tissue extracts causes a temporary augmentation of intestinal peristalsis, probably secondary to the disturbance of blood supply that occurs as a result of the fall in blood pressure. Magnus (326) and Enriquez and Hallion (327) also observed that extracts of the intestinal wall and mucosa increased the activity of isolated intestinal segments and of the motility of the intestine in intact animals. Zuelzer, Dohrn, and Marxer (328) as a result of their work suggested that a specific intestinal motor hormone was elaborated in the intestinal mucosa and stored in the spleen. Dittler and Mohr (329), Schlagenweit (330) and Sabatovski (331) found that the intramuscular injections of such extracts had little or no effect on intestinal movements and that their intravenous injection caused toxic symptoms typical of most tissue extracts. Weiland (332) reported that if the intestine of living animals were bathed with warm Ringer's solution, the intestine gave off something to the solution which excited isolated intestinal strips to contract and which was antagonized by atropine. Le Heux (19) confirmed Weiland's experiments and came to the conclusion that the active principle was either cholin or some closely allied substance. He proposed the theory that cholin is the gastro-intestinal hormone and acts by sensitizing Auerbach's plexus, atropine decreasing motility by antagonizing cholin. Abderhalden and Paffrath (333) found free cholin in the intestinal wall; and that the amount in an isolated segment was proportional to the activity of the segment. Le Heux tried the effect of intravenously administered cholin (10 mgm.) on gastro-intestinal motility in intact animals. He observed a slight decrease in gastric emptying time (15 minutes), but his data do not warrant a positive conclusion. Kuhlewein (334) and Arai (335) reported that the intravenous administration of from five to fifteen milligrams of cholin was of value in the ileus due to chloroform or experimental peritonitis. Clinically, cholin has been tried in post-anesthesia cases and gastro-intestinal disorders with negative or variable results (336) (337). Mulinos (338), using intact, unanesthetized animals, found that injections of cholin caused only inhibitory effects. Carlson, Smith and Gibbins (339), using barbitalized dogs, observed that the action of cholin on the motility of the stomach and intestine was quite variable, and concluded that it was difficult to interpret their results on the theory that cholin is the normal stimulus to motility.

If there is a gastro-intestinal motor hormone, it is going to be very

difficult to demonstrate conclusively its existence. Ivy, Farrell and Lueth (165) were unable to observe any definite increase in the motility of a subcutaneously transplanted jejunal loop after the ingestion of a meal, although the transplanted loop manifested a spontaneous motility at irregular intervals and became very active when its mucosa was mechanically or chemically excited.

Gastrin and secretin on motility. Gastrin (crude) (340) given subcutaneously in doses sufficient to cause copious gastric secretion does not affect gastric motility. The effect of purified secretin on motility has been discussed above.

Fat inhibition of the stomach. A chalone? Fat inhibition of gastric motility is generally ascribed to a nervous reflex. However, Ivy and Farrell (341) while studying the motility of a transplanted gastric pouch found that fat given by stomach tube was causing inhibition of the motility of the transplant after a latent period of only five minutes, whereas protein and carbohydrates did not cause inhibition until after a longer latent period (ten to twenty minutes). This proved that the inhibition of motility was at least partly humoral in nature. They suggested that the rapid inhibition of the transplant by fat was due either to a chalone (inhibitory autacoid) or to the rapid absorption of some volatile constituent of the fat introduced into the stomach. Bercovitz (342) observed inhibition of a "denervated" Heidenhain pouch on the introduction of food containing fat into the main stomach. Robins and Boyd (343), working with two "denervated" Heidenhain pouches, found that in one olive oil in the main stomach caused inhibition of the contractions, but in the other the "denervation procedure" abolished all rhythmical contractions. Irregular contractions occurred, however, which were not influenced by olive oil in the main stomach.

The possibility that fat inhibition may operate on the principle of the "humoral transmission of nerve impulses" as postulated by Brinkman and Van Dam (344) or according to the phenomena (vagusstoff) described by Loewi (112) (26) (345) must be kept in mind, but probably does not operate because it is still questionable whether such phenomena occur in normal animals or can occur when blood is used as the perfusing fluid.

Further work is necessary to clarify the mechanism of this inhibition.

EFFECT OF OTHER ENDOCRINE GLANDS ON GASTRO-INTESTINAL MOTILITY. *Parathyroids:* Carlson (346) found that in severe tetany following removal of the parathyroids, any deviation from the normal in the motility of the stomach and intestines is in the direction of depression

or paralysis. Dragstedt (347) on reviewing the literature concluded that "the disturbances in the digestive tract are therefore an effect of the tetany and are not directly due to a decrease in the amount of parathyroid hormone liberated in the blood." The effect of a parathormone on motility has not been studied.

Thyroid: It is a matter of common knowledge that patients with hyperthyroidism may have gastro-intestinal motor complaints, and that the feeding of thyroid extract to man and animals frequently causes diarrhea. Lorrenzi (348) reports that the thyroid feeding markedly increases the rate at which the stomach empties. However, the mechanism that is involved in these effects is not known, no serious attempt to analyse it having been made. A number of factors is probably concerned, e.g., the toxemia, increased metabolic activity of the intestinal musculature, the direct exciting effect of the active principle on the nervous mechanism of either the extrinsic or intrinsic nerves, the disturbance of digestive secretions, etc.

Adrenals: The effect of adrenalectomy on gastro-intestinal motility has not been studied. The anorexia that is known to result suggests depression of gastric motility, and the hemorrhages into the muscoa of the bowel that occur would certainly greatly disturb intestinal motility. Hoskins and McClure (349) found that intravenous epinephrine in etherized dogs causes depression of intestinal motility in doses smaller than those required to raise the blood pressure. This was confirmed by Durant (350) for dogs and cats, but in rabbits it was found that the blood pressure could be raised by epinephrine without significant effects on the intestine. Dragstedt and Huffman (351) found that in dogs under anesthesia epinephrine caused the effects on the intestine noted by the above workers, but that in normal dogs under morphine or paraldehyde small doses of epinephrine (1.0 cc. of 1 to 1,000,000 per kilo per minute) would cause slight rises in blood pressure without inhibiting intestinal motility, which shows that one of the objections to the old tonus theory of medullisadrenal function, namely, that blood pressure effects could not be obtained except at the price of intestinal paralysis, can no longer be maintained.

Insulin: In extirpation diabetes it is known that an increase in gastro-intestinal motility occurs (352) (132). Part of this is due to the disturbance of intestinal digestion (132). Part of it is due also to the disturbance of sugar metabolism as is shown by the work of Bulatao and Carlson (353) and Quigley and Carlson (354). These workers find that insulin causes increased motor activity of the duodenum and colon in

normal men and dogs, which is abolished by glucose administration. Section of the vagi, atropine, and epinephrine reverse the action of insulin on the stomach. In diabetic dogs, Bulatao and Carlson found that insulin causes a primary depression of gastric tone and hunger contractions which is followed by an increase in motility when the initial stage of hypoglycemia is reached, and that glucose given to diabetic dogs does not inhibit hunger motility unless insulin is given. They suggest that under ordinary conditions hunger motility increases parallel with the reduction of the tissue glycogen, and an enforced energy metabolism of lipoids on the part of the motor tissues of the stomach. However, the fact that vagus section reverses the action of insulin makes it appear as if its effect is due to the action of hypoglycemia on the nervous centers involved in the regulation of gastro-intestinal motility. It is evident, then, that the hormone, insulin, through its effects on metabolism plays an indirect rôle in regulating gastro-intestinal motility.

SUMMARY

It is evident that hormones or autacoids play an important rôle in digestion. Further progress of a conclusive character awaits and depends to a great extent on the discovery of chemical methods for the isolation of the various active principles, so that the mechanism of their production and specific action can be studied and correctly evaluated.

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THE MECHANISM OF THE SENSE ORGANS

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In the past four years the use of the thermionic amplifier has made it possible to detect the impulses in individual nerve fibres passing to and from the central nervous system. The method has been used mainly in the study of the sensory discharges and the aim of this review is to discuss the response of the sense organ to excitation and to relate its behaviour to that of other excitable structures such as the nerve or muscle fibre. Before this can be done some details of technique must be mentioned if only to show why so many problems have still to be solved.

LIMITS TO AMPLIFICATION. The operation of the thermionic valve as an amplifier of small potential changes is too well known to require description and its application to the special problems of physiology has been considered in detail in papers by Forbes and Thacher (1), Daly and Shellshear (2), Gasser and Newcomer (3), Chaffee, Bovie and Hampson (4), Adrian (5), Rosenberg (6), etc. There is, however, one point which may be mentioned as it shows what can and what cannot be done by amplification. With any well designed amplifier it should be possible to translate very small and rapid changes or potential in the input circuit into very large potential or current changes in the output, and within limits, which can be varied at will, the enlarged version should be a faithful copy of the original. The amount of amplification possible is limited by two factors. At the output end the characteristics of the final valve in the chain will determine the magnitude of the current fluctuations which can be produced without distortion; this factor was originally of some importance, but the more modern valves can handle such large currents that almost any demands for greater power could be met without much difficulty. In fact the sensitive recording instruments originally used have been abandoned in favour of systems chosen entirely for their ability to follow very rapid changes, the most suitable instrument for work on the sensory discharge being the moving iron oscillograph of Matthews (7). But although in theory the very smallest input changes can be amplified into large output changes, there is in practice a fairly definite lower limit to the magni-

tude of the input changes which can be dealt with usefully. These changes are applied to the grid of the valve and produce fluctuations in the current carried by the stream of electrons from the filament to the plate. Unfortunately the emission of electrons from the filament is not absolutely steady and there are very small and rapid fluctuations in the current apart from any changes in grid potential. Unless the changes impressed on the grid are appreciably larger than these internal fluctuations, we cannot expect to separate them, whatever the subsequent amplification, and at present it does not appear possible to deal with input changes smaller than about 2 microvolts. For recording the impulses in most nerve fibres a sensitivity of this order is quite large enough, for under suitable conditions the action potential due to a single fibre in a very small nerve may reach 50 microvolts or more, but the limitation might well become a serious difficulty in investigating the smallest non-medullated fibres.

PREPARATIONS EMPLOYED. With any system capable of showing rapid potential changes of the order of 5 microvolts or more it should be possible to record the action currents which occur in a sensory nerve fibre when the sense organ is stimulated, but it has to be remembered that a system as sensitive as this is liable to be affected by all manner of electric disturbances. Screening the preparation in a metallic case will protect the input from electromagnetic oscillations due to unsteadiness in the mains, etc., but the preparation itself may be the source of disturbances, for currents are generated whenever a muscle contracts or a nerve conducts, and the electrode system must be arranged to minimize the effects of these in comparison with the action currents from the particular nerve fibre under investigation.

When we are dealing with with a muscle instead of a nerve it is possible to lead off from small groups of muscle fibres by a needle electrode system with the two poles set very close together (Adrian and Bronk, 8) and the range of such a system may be so restricted that the muscle can be left in situ and the electrodes merely thrust into its substance. With a nerve, however, the effects developed at the electrodes are so much smaller that it has been impossible up to the present to use a needle system buried in the tissues, and the nerve must be freed from its surroundings and suspended in air between two electrodes at least 1 to 5 mm. apart. With such a small distance between the electrodes the action potential can never reach its full value: a greater separation may be necessary if the nerve contains more than a few hundred fibres, for the effect produced by a single fibre becomes smaller and smaller as the size

of the nerve trunk increases. It is therefore very much more difficult to analyze the discharges of the short, thick nerves from the special sense organs and most of our information concerns receptors in the skin and muscles.

Three methods are available for restricting the activity to a single fibre in the nerve. The simplest is to use a preparation in which the stimulus can be made to affect only one sensory ending. The discharge of the Pacinian corpuscle can be studied in this way (Adrian and Umrath, 9), for here the organ is large enough to be seen without magnification. In some parts of the skin a single hair can be moved with a fair chance of leaving the rest unaffected. Again the most complete data yet available on the discharge from a single organ have been obtained recently by Matthews (unpublished) using a small toe muscle of the frog supplied by a branching nerve, one twig of which connects with a single muscle spindle. The other methods consist in destroying all but one of the sense organs likely to be affected, as was done by Adrian and Zotterman (10) in the frog's sterno-cutaneous muscle, or in cutting through all but a few fibres of the nerve trunk. The latter method was originally used for investigating discharges in motor fibres, but Bronk (11) has applied it successfully to the sensory fibres in the frog.

It is often unnecessary to carry the process of elimination to the stage at which only one sense organ or sensory fibre is left in action, for discharges occurring simultaneously in two or three fibres are not too complicated to analyze. In many problems of an anatomical character it may be essential to keep all the sense organs and nerve fibres intact, e.g., when the sensory discharge is used to map out the area of distribution of a nerve or to trace the course of fibres from a particular class of receptor.

General nature of the sensory discharge. With every sense organ so far investigated the message carried by the individual nerve fibre is a succession of impulses identical in every way with those produced by electric stimulation of the nerve trunk and studied by the classic methods of electro-physiology. They appear as monophasic or diphasic potential changes according to the disposition of the electrodes; they are conducted at rates which agree well with Gasser and Erlanger's measurements for sensory fibres stimulated electrically, and their magnitude and rate of rise and decline are just what we should expect them to be. There were many good reasons which made this finding almost inevitable, but direct evidence is preferable to indirect and it allows us to argue more freely from the "artificial" to the "natural" product.

Thus we should expect to find that the magnitude of the sensory impulses is not affected by changes in the strength of the stimulus and there is, in fact, the familiar all-or-nothing relation as with electrically produced impulses. Again the phenomena of the refractory state ought to limit the sensory discharge to a series of discrete impulses with pauses in between, and we find no evidence of anything like a continuous activity in the sensory fibres.

Thus the content of the sensory message—the information which it can give to the central nervous system—is severely limited by the unvarying nature of the nerve impulse. A nerve fibre cannot conduct all manner of changes like a telephone wire; it cannot even conduct the dots and dashes of a Morse code. As a rule, with a steady stimulus, the impulses in a sensory discharge are evenly spaced and the only variable is their frequency which gives a measure of the intensity of excitation in the sense organ. This is all the information which a single fibre can convey, but as there are a million or more sensory fibres entering the spinal cord in man, we need not be surprised at the range of our sensations.

THE MECHANISM OF EXCITATION. The uniform pattern of the messages in each fibre makes it often a very difficult matter to analyze the complex discharge of a nerve trunk, but it shows that we have to deal with a fairly simple type of reaction and with one which is common to all sense organs, or rather to all the sense organs investigated hitherto. The reaction does not merely consist in a rhythmic discharge of impulses in the sensory fibre, for an important feature of the discharge is its decline under constant stimulation. It is this feature which helps us to draw comparisons between the sense organ and other excitable structures. The rate of decline varies from one sense organ to another and so allows us to arrange the various types in a series, and if this series were extended still further we should find that at one end would come the nerve fibre itself.

As examples of the sensory discharge we may take that from a hair and that from a muscle, since these two are at opposite ends of the scale. When a hair is bent, impulses pass up the sensory fibre only during the actual movement of the hair and there is no further discharge when the movement ceases, although the hair is maintained in an abnormal position. When a muscle is stretched, the sensory discharge reaches its maximum frequency during the period of extension, but if this is afterwards maintained at a fixed value the discharge continues at a lower frequency which declines very gradually. Here, as with the hair, the

maximum frequency depends on the rate of the stretching; after the movement has ceased the frequency depends on the amount of stretch and the time since the beginning of stimulation.

It seems, then, that the deformation of the end organ which constitutes the stimulus has the greatest excitatory effect whilst the change is in progress and that the effect dies away more or less rapidly when a ready state is reached. Since the factor which causes the decline is not equally developed in all sense organs we may consider what would happen if it were even more prominent than in the hair receptor. We should then find that a movement would not excite at all unless it exceeded a certain rate and that there would never be more than one or two impulses set up. Evidently the behaviour of a sense organ of this type would differ very little from that of a nerve fibre stimulated by a current which rises to a constant value. The current must increase at more than a certain rate if it is to excite at all and only a single impulse is produced by the change to new conditions. In the hair receptor the change produces ten or more impulses and in the muscle tension receptor it may produce thousands, but the declining effect of steady conditions is clear enough in both.

It is usual to regard the failure of a current to set up more than one impulse in a nerve fibre and the need for a liminal gradient of current increase as due to a rapid adaptation of the fibre to the new conditions. Various suggestions have been made as to the nature of the adaptation process; that it is due to a gradual leakage away of the ions concentrated by the current, to an opposing reaction which stabilizes the fibre, or to the depletion of a store of active material which breaks down slowly or rapidly according to the concentration of ions and to the amount of active material still available (Hill, 12). Although there are not yet enough data to decide the question, it is clear that we have to do with something which reduces the excitatory effect of the stimulus so quickly that it can only be effective during the initial period when the conditions are changing rapidly, and this something comes into play whether an impulse is discharged or not. With the receptor organ there is another possibility which must first be excluded if the comparison is to hold, namely, that the decline in the discharge may be due to fatigue. By fatigue is meant a change consequent on the activity of the organ as distinguished from one directly consequent on the stimulus and independent of the amount of activity it has evolved. Bronk (13) has shown that true fatigue effects most probably occur towards the end of a long discharge from a tension receptor, but the initial decline is evidently due

to a direct adaptation to the stimulus, for the frequency at any moment is determined by the rate of rise and the duration of the stimulus and not by the total number of impulses which it has set up (Adrian and Zotterman, 14; Adrian, 15).

All this does not prove that the adaptive change is the same in the sense organ as in the nerve fibre, but at any rate there is a close enough likeness to suggest that we are dealing with a common type of excitatory mechanism in both. Now we have already a reasonable working hypothesis of the processes which cause an impulse to travel down a nerve fibre after an electric stimulus. This is the "membrane theory" as put forward by Bernstein and Lillie, and according to it an excitation is caused by a change in the surface layers of the fibre. These are normally polarized and impermeable to certain ions: the stimulating current depolarizes the surface at the cathode with the result that this region becomes "active", and the activity causes a movement of ions which has the result of depolarizing the surface further on. This in turn becomes active and leads to a further spread of the depolarising and so the active region travels down the fibre accompanied by the electric changes which give rise to the action current. As long as the fibre is uninjured the activity at any point is only momentary, for the surface reverts almost at once to the resting, polarised condition, and until the fibre has passed through the refractory state it cannot be reëxcited. For a more detailed account the papers of Lillie (16) and of Bishop, Erlanger and Gasser (17) should be consulted and in particular the recent paper of Bishop (18) dealing with objections raised by Craib.

The attempts to supply a more detailed picture of the excitatory process in nerve have naturally to take into account the rapid adaptation which prevents a constant current from discharging more than one impulse. In the discharge of a sense organ we are dealing with a succession of impulses set up by a continued stimulus, but there is no need to introduce any fresh conception to account for this, for it would be the normal consequence of a slow rate of adaptation. Quite recently two observations have been made which supplement one another in a remarkable way and show that under suitable conditions, a region which remains permanently in the active or depolarised state will in fact give rise to a series of impulses. The first of these relates not to the nerve fibre, but to Lillie's iron wire model which has done so much to win acceptance for the membrane theory. Lillie (19) finds that when an iron wire containing very little carbon is placed in nitric acid of the right strength, a rhythmic series of activations may spread down the wire

from a region which is permanently active. Such a region is produced by local conditions which restrict diffusion so that the acid immediately surrounding the wire becomes too weak to reinstate the passive layer. The steady current set up between the active and the adjacent passive surface causes the latter to break down rhythmically, the rhythm being determined by the refractory period following each activation. More recently the writer has found that in mammalian nerve fibres which have been cut through a discharge of impulses at a high frequency (400–150 a second) may continue for many minutes at a time. The cut end of the fibre is permanently active in the sense that it remains permanently at a negative potential with respect to the uninjured region, and there is evidence to show that the discharge is due to repeated excitation by the current from the injured point. As we should expect, the chief condition for the appearance of a repeated discharge is that the nerve fibre should be unable to adapt itself to the stimulus at the usual rate. The small medullated fibres are the most prone to react in this way, but even the motor fibres may be induced to do so by substances which alter the osmotic equilibrium of the fibre. An account of this work will be published shortly; for the present discussion its interest lies in the fact that the discharge from the injured end of these nerve fibres bears a remarkable likeness to that from a sense organ excited by a constant stimulus. It is true that the sense organ can vary the rhythm of its discharge according to the intensity of the stimulus, and the frequency is on the whole a good deal lower than that from a cut fibre, but in other respects, e.g., the degree of regularity and the failure of the rhythm at the end of a discharge, the likeness is too striking to be set aside.

Until we know more of the excitation process in a nerve fibre there would be little value in attempting a detailed theory of receptor excitation. But there is enough evidence to make it highly probable that the determining event in the nerve ending as in the nerve fibre is a change in the properties of the surface layer, with a resultant depolarization and a flow of current which will produce a periodic activation of the nerve fibre. In those sense organs which respond to mechanical deformation the most obvious effect of the stimulus is to bend or stretch the naked terminations of the axis cylinder. The consequent thinning of the surface layers might well lead to changes in permeability and polarisation great enough to excite, but not equal to the complete depolarisation produced by cutting the fibre. In this way graded stimuli might produce different degrees of activity at the ending and different rates of impulse discharge. In fact, Lillie's rhythmically active wire could be made into

an excellent model of a sense organ, for the rate of discharge can be varied over a wide range by altering the condition of the active region which produces the steady current. It is, however, unnecessary to suppose that the sensory ending has any region which is kept in a state of continuous activity by the stimulus, for all that is required is that the region should revert to the active state after each impulse has been discharged.

FREQUENCY OF DISCHARGE. It has been stated above that the frequency of discharge from a sense organ is usually lower than that from the cut end of a nerve fibre and this statement needs some elaboration. As we should expect, when a nerve fibre of the unadapting type is cut through, an impulse is discharged whenever the fibre has recovered sufficiently to respond to the stimulus furnished by the injured area. In a mammalian fibre at body temperature the whole process of recovery is practically complete in 5 to 8 σ and in conformity with this we find that the frequency of an unbroken series of impulses is seldom less than 150 a second and is usually between 400 and 200 a second. With the sense organ discharge it was thought originally (Adrian and Zotterman, 20) that the frequency was always so low that the nerve fibre would have time for complete recovery between each impulse. Matthews has shown that this is incorrect, for with rapid extension a frog's muscle receptor may discharge at a frequency as high as 200 to 300 a second for a short period, and this may well occur in life when a rapid movement is carried out. With slower extension, however, and in the later stages of any discharge when the adaptation has asserted itself, the frequency is very much lower, and values of 60 to 25 a second are commonly found both in frog and mammalian receptors. For such low frequencies to occur we must suppose that the stimulus is acting on a structure which recovers its full excitability at a much slower rate than the nerve fibre, though the high initial frequencies show that there is very little difference in the earlier stages of recovery—unless we suppose that an intense excitation produces a continuous instead of an intermittent activity at the nerve ending.

Thus there are differences between the sensory ending and the sensory nerve fibre both in regard to adaptation and rate of recovery, but we should expect to find quantitative differences between the reaction of an axis cylinder covered with a myelin sheath and that of the naked terminal branches. What is more remarkable is that qualitative differences are absent, or rather that the only qualitative difference is that the nerve ending can be excited by changes in its environment which

have no effect on the nerve fibre. Even in this, which, to be sure, is the main characteristic of the sensory ending, the distinction from a nerve fibre seems to be a matter of degree only. Mechanical stimulation will excite a fibre if intense enough, though the excited region will then remain permanently depolarised instead of recovering like the sensory ending. Heat will also excite with the same consequences. Cold is, at first sight, a more difficult problem, for cooling is not usually regarded as a possible stimulus to a nerve fibre.[1] But it is interesting to find that a comparatively small rise or fall of temperature will often start a serial discharge from the cut end of a mammalian nerve fibre of the small medullated type; in fact, we can sometimes manufacture an excellent artificial temperature organ by the simple process of injuring a nerve fibre.

It would be a mistake to insist too much on the simplicity of the excitation process in the sensory ending, for all that can be said at present is that it is not far different from that in the nerve fibre, and this can only be described in the most general terms in spite of the vast amount of work which has been spent on it. Only one thing is known about the metabolism of the sense organ and that is that oxygen plays the same part in its activity as it does with other active tissues, for Bronk (21) has shown that the tension receptor in frog's muscle can discharge in the absence of oxygen but that fatigue is then more rapid, and this fatigue disappears when oxygen is readmitted.

There is some temptation to go further afield by likening the discharge from a sense organ to that from a motor nerve cell. It is certainly an easy matter to choose records of sensory and motor fibre discharges which are quite indistinguishable (Adrian and Umrath, 22), for the frequency of the motor discharge in each fibre ranges between 5 and 100 a second. But again there are quantitative differences, for the very high frequencies which can be obtained from sense organs seem to have no counterpart in the motor discharge There is also an important qualitative difference, for the rhythmic activity of the motor neurone is controlled by a balance of excitatory and inhibitory effects and so far the inhibitory effect is without any obvious parallel in the sensory discharge.

THE DISCHARGES FROM DIFFERENT TYPES OF SENSE ORGAN. The common make-up of all sensory discharges has been discussed at such

[1] This statement needs some reservation. Cooling will not excite motor fibres, but cooling the ulnar nerve at the elbow is known to give rise to pain referred to the distribution of the nerve.

length that little room is left for considering their differences. This may be all to the good, for there is nothing very definite to consider. Differences in adaptation rate have been mentioned already and there is no doubt of their existence, although it is not easy to obtain strictly comparable results from different types of receptor. The writer has pointed out elsewhere (23) that the more complex, encapsulated organs seem on the whole to have a slower adaptation rate than the free nerve endings. Certainly the receptors in the frog's skin become adapted very rapidly under a constant deformation and so do the receptors for hair movement in the cat, whereas the Pacinian corpuscle and the muscle spindle give persistent discharges. The only other clear case of a receptor giving a persistent discharge is found in the lung, for as long as the lung is distended impulses continue to pass up the vagus. The receptors are in the lung root and this is known to contain complex sensory endings, though there is no evidence of a capsule surrounding them (Berkley, 24). But it is on the whole unlikely that there is any essential connection between the structure of the end organ and its rate of adaptation. The sense organs responsible for postural adjustments have to keep the central nervous system constantly informed of the relations of the different parts of the body and to do this their messages must be not only constantly maintained but very accurately graded as well. A complex encapsulated structure may be important as much for the accurate gradation as for the persistence of the message, for the capsule will protect the nerve endings against casual changes in environment and the whole structure seems well adapted for producing a graded deformation of the endings in response to graded mechanical forces. Again, the fact that mammalian nerves contain fibres which adapt very slowly to the stimulus of an injured region makes it improbable that the free nerve endings are all of the type which adapt rapidly. Indeed, if some of these endings or the fibres connected with them are responsible for pain, we could often wish that the rate of adaptation were a great deal quicker.

Another and more definite correlation between structure and function has been made by Bishop, Erlanger and Gasser (25), though it relates to the structure of the sensory fibre rather than the sense organ. By analysing the total response of a nerve trunk to electric stimulation they have shown that the sensory fibres from muscle are larger and conduct more rapidly than those from the skin, and that sensory fibres in general differ widely in conduction rate and in the intensity (at the electrodes) and rate of rise of the action current, according to the diameter

of the fibre. Matthews (26) has confirmed the difference in the impulses from frog's muscle and skin, using end-organ instead of nerve trunk stimulation and recording the action currents in individual nerve fibres. Gasser and Erlanger suggest that the characteristic time relations of the impulses in different fibres may determine their fate in the central nervous system owing to the existence of intra-medullary receptors tuned to respond preferentially to impulses of a particular form. This seems to involve the assumption that there are characteristic differences not only in the diameter of the fibres, but in that of their terminations as well. It should be noted, however, that there is no question of one and the same nerve fibre being able to conduct two different kinds of impulse, for the form of the impulse is determined by the size of the fibre at each point and not by the quality of the stimulus. Their work makes it clear that different types of sense organ are supplied by nerve fibres of different size and that the impulses in these fibres differ in form. If the fibres from different sense organs and from different internuncial tracts in the cord meet somewhere at a common point, the path taken by a particular message could only be decided by the characteristic form of the impulses. Whether there are such common points is an open question; the fate of the message must depend in part on the particular distribution of the nerve fibre terminations and the more restricted these are the less need would there be for a specific tuning of receptive cells in the cord. But it is quite likely that the fibres from receptors of different type may arborise widely over a number of cells and that the order in which these cells are affected by messages of increasing intensity will depend on the form of the impulses reaching them. In any case, Gasser and Erlanger's work gives us an extremely valuable means of identifying the size of nerve fibre concerned in a particular discharge and it has already been put to good use by them.

It will be apparent that most of our information on the working of the sense organs has come from those which respond to mechanical stimuli and that little has been said about the temperature organs. The difficulty is that injured mammalian fibres which have nothing to do with temperature sensations will often start discharging when they are cooled or warmed, and such discharges may mask anything from the temperature organs themselves. The same difficulty arises in connection with pain, and for the present the whole subject of pain endings and pain fibres must be left until more evidence is available. With this we must also leave the possible differences between protopathic and epicritic receptors and many other points which are the main problems usually discussed in connection with the physiology of the sense organs. All

that can be said is that so far the different sense organs all seem to work on a common plan and this is true of invertebrate as well as vertebrate organs, for Cunliffe Barnes has found sensory discharges of the usual type in the nerve of the crab's leg, although the fibres are non-medullated and conduct very slowly. We may expect to find quantitative differences such as varying rates of adaptation and varying relations between stimulus intensity and discharge frequency and we may be able to relate these to the structure and function of the sense organ concerned; but it is exceedingly unlikely that any organ produces a discharge of an entirely different type, for the simple reason that the discharge must be conducted by a nerve fibre and all that this can conduct is a succession of impulses more or less widely spaced.

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